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MILITARY BLOOD BANKING

IMMUNOHEMATOLOGY FOR THE REFERENCE AND FORENSIC TESTING LABORATORY

A MONOGRAPH

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15 September 1971

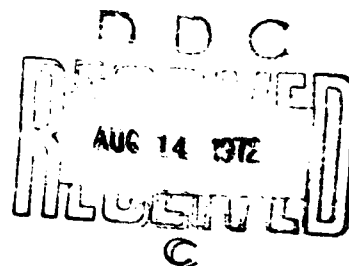
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A MONOGRAPH

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IMMUNOHEMATOLOGY

Introduction

In this monograph, important problems and pitfalls involved in whole blood and blood component therapy will be discussed. Some aspects of immunohematology will be emphasized; however, the central theme will be that of preventing injury from blood transfusion.

Medicolegal Problems (1)

Four major points will be considered.

1. Immunity of hospitals and blood banks.
2. Hemolytic transfusions reactions.
3. Transmission of hepatitis.
4. Injuries to blood donors.

Immunity of hospitals and blood banks. In several jurisdictions, hospitals and blood banks remain completely immune from liability for harm proximately resulting from the negligence of their employees or agents. Even in such jurisdictions, the advisability of carrying insurance against liability should be considered. There are exceptions to the doctrine of absolute immunity, but more serious is the threat posed by the growing number of courts that have been willing to overrule the doctrine of charitable immunity, thus placing hospitals in the same legal position as noncharitable institutions. When a court rules in favor of the plaintiff, the decision is usually retroactive. (The Supreme Court of Michigan made its ruling prospective only as to all charities except the one being then sued.) If the ruling is retroactive, any claim arising within the period of the statute of limitations can be brought before the court.

The majority of the courts seem convinced that the doctrine of charitable immunity is unsound, and that none of the historical arguments on which it is based can stand analysis. The conflict among the jurisdictions arises over whether the doctrine should be overruled by a court, or whether this is a legislative function. The New Jersey Court has said, "When a principle of law no longer serves justice it should be discarded," and overruled its charitable immunity rule in 1958. It is important to note that the New Jersey Legislature restored the rule by statute. Also in 1958, the Supreme Judicial Court of Massachusetts said "While as a general proposition the doctrine might not commend itself to us today, it has been firmly embedded in our law for over three quarters of a century and we think its 'termination should be at legislative, rather than judicial, hands.'"

Many jurisdictions have rules which grant partial immunity to charities. These rules differ greatly in detail. Similar to the rule granting general immunity, rules granting partial immunity appear to be crumbling before the broad criticism of the charitable immunity doctrine.

Hemolytic transfusion reactions. Scientific and medical expert testimony in any case involving injury to a blood donor or to a transfusion recipient is crucial. Not only does it establish the standards of professional practice against which the particular conduct of the hospital personnel will be judged, but it serves as the technical factual background that will influence the court in choosing among various possible applicable rules of law.

The courts have been willing to accept evidence that a patient transfused with incompatible blood--such as B blood to an O patient, or Rh positive blood to a sensitized negative patient--is *prima facie* evidence of negligence, and of sufficient weight to assure a trial by jury.

No court appears to have held that an hemolytic reaction in a transfusion patient alone is adequate enough to constitute a *prima facie* case of negligence. Where the argument has been raised by plaintiff's counsel that *res ipsa loquitur* should apply, some courts have accepted medical testimony that an hemolytic reaction may occur without negligence, and refused to apply the doctrine. On occasion the defendant has asserted that no hemolytic reaction occurred, and a decision in his favor upheld. Judicial respect has also been given to testimony that an hemolytic reaction might be due to a hidden factor impossible to detect, and hence explainable as an unavoidable accident. We can expect that some courts, drawing on statements like those of Dr. Wiener that "the techniques for typing blood and checking the findings are about perfect," and that "most of the deaths result from human error," will hold that evidence of an hemolytic reaction following a transfusion constitutes *prima facie* evidence of negligence, or that *res ipsa loquitur* applies. Either rule would afford considerable aid to a plaintiff in establishing his case.

The medical literature emphasizes that many errors occur during situations when the hospital is understaffed or incompletely trained personnel are on duty, particularly on weekends or holidays. The importance of reliable, well-trained technicians and other personnel--and the need for extreme care in situations in which such personnel are not available--cannot be overemphasized. Training of personnel and maintenance of rigid standards of performance are essential.

It would probably constitute negligence to administer a transfusion, in the absence of an emergency situation, without first making the minimum routine grouping and crossmatching standard tests (JAMA, 169: 1380, 1959.) Wiener suggests that only in an emergency should universal donor blood be used, and that even then appropriate notations should be made in the patient's chart (JAMA, 156: 1301, 1954.) The courts ordinarily test the adequacy of a procedure by the acceptable standards in that area; but

a court might hold that such standards lagged unreasonably behind sound accepted scientific technique.

Transmission of hepatitis. Recent terminology refers to tests for detection of hepatitis and include:

Hepatitis-Associated Antigen Test (HAA Test)

Serum Hepatitis (SH Test)

Australia Antigen (Au Antibody Test)

Late in 1970, the National Academy of Sciences, National Research Council, appointed an Ad Hoc Committee to review Hepatitis-Associated Antigen (HAA) tests.

The committee recognized that the detection of HAA in a person's serum appears to be a specific indication that the person is a carrier of one of the causative agents of human viral hepatitis. It is also acknowledged that even when the test is properly conducted, currently available tests for HAA identify only a portion of the donors whose blood will transmit viral hepatitis; at best, it detects 20-25% of carriers.*

The committee concluded that when methodologic, supply, and licensure problems are solved, all donor blood should be tested for the presence of HAA. It further concluded that all blood banks that have the required capability and resources should be encouraged to begin testing (3).

Several technics have been developed for testing, including complement-fixation, gel diffusion, immunoosmoelectrophoresis (IEOP), hemagglutination inhibition, and a radio immunoassay for Australia antigen which recently has been described (2).

As this monograph goes to press, additional methodologies are appearing rapidly with claims of HAA carrier detection as high as 80%. Although much research remains to be done in this field, and despite the hectic rush to implement testing in all blood bank centers, we should witness a decrease in the transmission of hepatitis through the transfusion of whole blood and blood components.

Injuries to donors of blood. The blood donor expects, and is entitled to, ordinary care, including the exercise of ordinary professional skill. This begins with the medical history to assure that donation of blood will not harm the donor. The hospital or blood bank probably has an affirmed duty to inquire as to the time of previous donation. It might constitute negligence to knowingly accept blood from a donor who has too

* As of March 1971.

recently made a previous donation. It has been suggested that postcoronary donors should be refused in order to avoid any possible implication that a donation was a precipitating factor in bringing on another attack (JAMA, 160: 1268, 1956). The hospital or blood bank may be held liable for injury resulting to a donor in the course of normal activities subsequent to the donation, in which a weakened condition resulted and the staff knew, or had reason to know, that the donor had to engage in strenuous activity, and failed to warn him of the possible danger.

Consent of the donor is required. A technical question might be raised of lack of consent of a minor donor, making the blood bank or hospital liable for battery. Local law should be consulted on this point. Some states hold that a minor is capable of giving consent if he is sufficiently mature to understand the significance of his actions. The procedure of the Southwest Blood Banks provides that the consent of the parent should be obtained before accepting an unmarried donor under age 21 and not in the military service; this appears to constitute wise and conservative practice.

Sound procedures for the preparation of the patient's arm and sterile precautions in blood-taking should be established and rigidly enforced. Recovery has been allowed based on failure to follow established and customary sterilization procedures. Where sound procedures have been established and followed, courts have denied recovery, arguing that the claimant failed to establish that the infection came from the hospital venipuncture rather than from another source (1).

General Considerations

In spite of meticulous care and close attention to procedural detail, it appears inevitable that a small number of undesirable transfusion reactions still occur. Among the several classes of transfusion reactions, those which produce intravascular hemolysis are the most dangerous and may cause the death of the recipient. For this reason, we have long accepted the responsibility for reexamination of tests on all patients in whom hemolytic transfusion reactions are suspected. In our zeal to close all possible avenues of error we have randomly evolved a time-consuming set of repetitive maneuvers and serologic tests that fail to lead quickly to answers that are necessary to care properly for these patients. There can be but little quarrel that the data required by the Committee on Standards, AABB (4), are useful. It would appear, however, that the first order of business is to provide a "yes" or "no" answer as quickly as possible to the simple question "Has an hemolytic transfusion reaction occurred?" An affirmative answer to the question means that the patient requires emergent treatment. Subsequently, the rechecks recommended by the Standards should be completed as promptly as possible, and may then be carried out without further compromising the recipient's chances of surviving an hemolytic reaction to transfusion.

The existence of Incompatible Hemolytic Blood Transfusion Disease (IHBTB) can be established quickly and accurately by demonstrating: first, a positive direct antiglobulin (Coombs') test, which appears during or after a transfusion; and second, the presence of elevated free hemoglobin level in the serum or plasma of the recipient.* In a recent appraisal of the diagnosis and treatment of IHBTB, Nalbandian et al (5) emphasize the need for a fresh approach to the problems of detection and treatment of victims of incompatible blood transfusions by clinicians and blood bank workers alike. In the interest of saving the lives of recipients receiving incompatible blood, these authors stress the need for speed, simplicity, and precision by a technique of cross-checking. They contend, and we agree, that the chances of survival vary inversely with the time interval between the infusion of incompatible blood and the start of treatment. Treatment with mannitol, in accord with the recommendations of Barry and Crosby (6) and of Parry (7), has been endorsed. Nalbandian and associates emphasize the need for speed, and propose that the simple visual inspection of posttransfusion serum is adequate to confirm significant hemoglobinemia. A direct antiglobulin test can be performed in a very few minutes on the same specimen of blood. We have thus fulfilled their criteria of speed and simplicity. Concerning precision: the diagnosis and treatment of incompatible blood transfusion disease and stocks of mannitol have been placed by Nalbandian and his associates at each nursing station throughout the hospital where blood transfusions may be given.

In our estimate, the salient areas to the avoidance of pitfalls concern:

1. Communication between personnel in the patient care area and the laboratory. This must be established from the moment a reaction is first suspected and maintained throughout the interval of observation and/or treatment.
2. Communication within the laboratory between bench workers and physician supervisor, irrespective of the hour or the day of the week a reaction is recognized.
3. Care with technique of obtaining samples required for the study.
4. Documentation of samples, including relationships in time to infusion of the offending transfusion and to each other.

As we have indicated above, once the diagnosis is established, treatment with mannitol must be prompt and directed toward maintaining urine output at approximately 100 ml/hr. With respect to technique, the single most significant pitfall to be avoided is the artificial induction of hemolysis in the first posttransfusion specimen of blood. This can be

* Crosby-Furth plasma hemoglobin technique.

prevented only through careful venipuncture following minimal stasis and probing, and by the use of chemically clean and dry equipment.

The clear designation of time and date, along with proper patient and source of specimen identification, is the only means by which accurate reconstruction of events can be achieved. Properly organized, the blood bank laboratory will have saved all crossmatch specimens and pilot tubes for a minimum of 7 days following transfusion. These specimens, along with samples from the container involved in the reaction and post-transfusion samples, now can be rechecked for groups, Rh type, and compatibility testing. When the evidence suggests that an hemolytic reaction has in fact occurred, antibody titrations before and after transfusion, especially the pertinent natural, regular isoantibodies, will provide confirmatory data. Culture of pertinent blood specimens should be performed. Quantitative haptoglobin estimates under certain conditions provide another parameter of evaluation of a delayed, confirmatory nature.

When other accidents appear to be responsible for an hemolytic transfusion reaction, e.g., gross bacterial contamination or massive hemolysis of a donor unit, whatever the cause, the sequence of communication and notification of top level professional personnel is even more important, because these patients may die in a very short time. For the latter problems, the nature of specimens and the way in which they are to be handled may, of course, be modified to fit the circumstances. The necessity for accurate documentation and labeling of materials to be studied remains the same. The method of Dr. Paul J. Schmidt (8), shown in Table 1, summarizes the findings and treatment of transfusion reactions.

TABLE 1

MANAGEMENT OF TRANSFUSION REACTIONS

Findings	Treatment
Urticaria only	Intramuscular antihistamines, resume transfusion if controlled
Fever, chills, etc. 1. Examine patient's blood for: intravascular hemolysis (plasma hgb.) extravascular hemolysis (Coombs test) 2. Examine donor plasma for bacteria	1. Stop transfusion 2. If laboratory tests are negative treat with antipyretics and sedatives. With positive findings start prophylactic treatment as below
Shock, hemoglobinuria, oliguria, bleeding	1. Maintain blood pressure with vasopressor 2. Maintain urine flow over 100 ml. /hr. a. Mannitol 25 grams intravenously b. Fluids 3. Replace specific deficits when indicated a. Fresh plasma, platelet-rich b. Fresh frozen plasma c. Fresh whole blood, if necessary 4. Antibiotics and hydrocortisone for septic shock

Since the occurrence of a transfusion reaction may represent a technical failure of the blood bank service, each instance must serve as a

teaching and reorientation exercise for all personnel in the department. It is only by this means that every staff member can dedicate himself to minimizing the risks of blood transfusion. This highlights the absolute necessity for maintaining continuous and effective training programs and/or workshops for all personnel engaged in blood grouping, blood banking, and blood transfusion practices.

Pitfalls

Modern concepts of medical care demand an ever-increasing volume of safe blood for transfusion. In striving to meet this demand, one finds no less responsibility imposed upon the military than has been placed upon the civilian medical facility. Thus, the complex and interdependent series of procedures for pretransfusion testing of blood evolved during the past two decades has become equally important in modern military and civilian blood banks alike.

Although there remain many gaps in our knowledge, human error continues to be the major cause of untoward reaction to blood. Instances of human error, furthermore, seem more often the result of clerical than technical mistakes. The selection of a specific unit of blood for a given patient is a decision bearing absolute finality akin to no other laboratory procedure. There simply is no way to recover the wrong unit of blood once the recipient has been infused.

Because the responsibility for the selection of blood rests entirely with laboratory personnel, it is our conviction that training and discipline in blood bank laboratory technique cannot be overemphasized. By intensive training we can expect to fill some voids in knowledge and to minimize the chance of faulty judgment. Through incessant practice and experience we may expect to reduce the chance of clerical mistakes.

General principles. Although some 15 independent blood group systems have been discovered during the past 65 years, we will emphasize only those having importance in transfusion therapy and in the management of obstetrical patients and their newborn infants. Our concern with these specific problems arises from the knowledge that some of these blood group systems have attributes which may cause serious injury or death of patients who require the administration of blood. We also wish to avoid sensitizing female recipients with blood group antigens capable of causing erythroblastosis, if they should later become pregnant.

As in all blood group systems, the scheme for designating the major ABO groups depends upon the antigen present on the erythrocytes. Thus, red cells of a specimen of group A blood contain the blood group antigen A, and cells of a specimen of group B blood contain the blood group antigen B. Similarly, blood which is Rh-positive has erythrocytes containing the antigen Rh₀. Red cells lacking the antigen Rh₀ are designated Rh-negative.

The presence of any given antigen on an erythrocyte may be demonstrated by the use of a blood grouping serum containing the antibody specific for the particular antigen. Under appropriate testing conditions, for example, an anti-A blood grouping serum will agglutinate red blood cells containing the antigen A. Because this agglutination can be seen, we recognize that some sort of reaction has occurred. Therefore, we infer that the anti-A antibody in the serum has attached itself to the A antigen on these cells and that agglutination resulted. When cells containing only the antigen B are exposed to an anti-A serum, no agglutination will occur. Under these circumstances, we must conclude that there is no antigen A on group B erythrocytes.

The testing of unknown cells with sera of known antibody content is referred to as direct or cell grouping. A positive cell grouping test indicates the presence of the antigen, and is recognized because agglutination occurs. Failure of an antiserum to agglutinate a sample indicates the absence of that antigen.

The results of red cell ABO grouping tests may be verified by determining which antibodies are present in the serum of the blood sample. This confirmation technique is known as reverse or serum grouping. It involves the use of three separate samples of erythrocytes known to contain antigens A, B, and O for testing the unknown serums. If an unknown serum agglutinates B but neither A nor O cells, one learns that anti-B antibody is present in that serum. There is an invariable reciprocal relationship between the antigens A and B and their respective agglutinins, anti-A and anti-B. For this reason, we expect to find anti-A antibody in the serum of group B persons, anti-B antibody in the serum of group A persons, both anti-A and anti-B antibodies in the serum of group O people, and neither anti-A nor anti-B antibodies in the serum of persons having group AB blood. Existence of this reciprocal antigen-antibody relationship permits reinforcement or proof of the validity of the results of the cell grouping tests. Instead of seeking the identity of an unknown antigen by use of a serum of known antibody specificity as was described for the cell grouping technique, reverse or serum grouping techniques seek the identity of any antibody that might be present in an unknown serum through the use of cells of known antigenic components. Now, the occurrence of agglutination indicates the presence of an antibody in the unknown serum which is specific for the antigen on the test cells. It follows logically, then, that the absence of agglutination tells us there is no antibody in the unknown serum for the antigen on that specific test cell. It is appropriate to mention that agglutinates resulting from serum grouping tests may be much less intense than those observed with cell grouping sera. The cause for these differences in intensity of agglutination will be discussed below (potency of the reagent, page 16). We have no similar built-in opportunity to verify results of cell grouping tests in any of the other blood group systems because the ABO system alone has naturally occurring isoantibodies, always reciprocal to the antigens present on the cells. While we are quick to acknowledge reports of "naturally occurring" antibodies in the Rh-Hr, Kell, I, Ss, Wra, and perhaps other

blood group systems, we prefer to stand on the conservative hypothesis stated above. Our reasons for this position result in part from the rare frequency of such examples as compared with the vast numbers of alternate experiences, and more importantly, with our inability to totally explain the existence of "natural" isoagglutinins even in the ABO system itself. As is the case so often in the practical application of serologic techniques, inconsistency may result from the inescapable need for arbitrary definition of terms. Thus, it may be argued that even the antibodies found consistently in the ABO system are, in fact, themselves immune and the result of environmental immunization of some kind. Experience has shown, nonetheless, regular presence of antibody in the ABO system that can and should be used for serum grouping confirmation tests. All antibodies specific for antigens of all the other blood group systems are irregular, and may be regarded as *de facto* evidence of prior immunization. The more commonly encountered irregular agglutinins are in the Rh-Hr, Kell, Duffy, or Kidd systems. Irregular antibodies have also been found for each of the remaining blood group systems including the low incidence or "private" blood group antigens that have so far been established. As a matter of interest, it is precisely in this way that the existence of a new system may first be suspected, and ultimately be proved. Table 2 shows the serological reactions upon which the ABO system is based.

TABLE 2

Serologic Classification of ABO System

Red Cell Grouping				Serum Grouping				Phenotype
Reactions with known sera			Antigen present on cells	Reactions with known cells			Antibody present in serum	
Anti-A	Anti-B	Anti-A,B		A	B	O		
+	-	+	A	-	+	-	anti-B	A
-	+	+	B	+	-	-	anti-A	B
+	+	+	AB	-	-	-	none	AB
-	-	-	O	+	+	-	anti-A,B	O

Symbol + indicates agglutination; - no agglutination.

Blood grouping reagents. Blood grouping reagents are sera. They are usually, but not always, of human origin and contain agglutinating antibodies capable of detecting specific blood group antigens. They are labeled according to the antibody they contain, e.g., "anti-A serum," which will agglutinate only cells bearing the blood group antigen A.

Reliable serologic results can be expected only if potent specific antisera are available, the proper technique in their use is employed, and the sera have been protected by proper storage conditions. Proper technique includes not only methods of mixing serum and cells, temperature and interval of incubation, and reading of results, but also the less obvious points of cleanliness of glassware, choice of control cells, and care to avoid contamination of antisera and test cells alike.

Subgroups of A. Blood possessing antigen A can be classified into two main groups. The 80% of A bloods that are agglutinated by a subgrouping serum (anti-A₁) are classified as A₁ (or A₁B). Those failing to be agglutinated by this anti-A₁ serum are classified as A₂ (or A₂B) or weaker, A₃, A₄, etc. These latter groups may be so weak as to even fail to be agglutinated by potent anti-A serums. These have been classified as A₃, A₄, etc.

About 1% of A₂ and one quarter of A₂B individuals have the irregular antibody, anti-A₁, in their serum. A proportion of A₁ and A₁B persons have the irregular antibody anti-H in their serum. These peculiarities are worthy of note, and can be a pitfall to the uninitiated. An excellent description of the subgroups of A weaker than A₂ (A₃, A₄, A_x) is presented in the thesis by Arne Gammelgaard.

Standards of blood grouping reagents. The Division of Biologics Standards, National Institutes of Health, has established minimum requirements for the manufacture and labeling of all of these reagents offered for sale in interstate commerce. These regulations apply to all such materials; thus, antisera must be sterile, nonturbid, have proper specificity, potency and avidity, and be free of homolysins, autoagglutinins and atypical antibodies. The avidity of a testing serum is a measure of its ability to agglutinate rapidly and completely. The criteria for test cells or other antisera will differ only in detail; Table 3 defines minimal requirements for anti-A and anti-B grouping sera.

TABLE 3

NIH Minimum Requirements
for
Anti-A and Anti-B Grouping Serum

Serum	Test Cells	Titer	Avidity* in Seconds
anti-A	A ₁	256	15
	A ₂	128	30
	A ₁ B	128	30
	A ₂ B	64	45
anti-B	B	256	15

*Term used to define the recognizable beginning of agglutination.

Reagent Red Blood Cells (Human). Over the past few years almost all of the commercial supply sources have introduced products known as Reagent Red Blood Cells (Human). These group O cells are from separate donors or prepared as pools of several donors, depending upon the use intended for the product. For the detection of irregular antibodies, pooled reagent red blood cells are offered commercially for routine screening of sera, although the use of separate cells has been demonstrated to be a more satisfactory technique. For the identification of irregular antibodies, sets of eight or more selected donor cells bottled separately must be used and are sold as "panels."

The National Institutes of Health (NIH) has moved cautiously in defining minimum standards for this class of blood bank reagents because of the complex problems posed by these products. While the use of preservatives is permitted, the manufacturer must demonstrate the absence of an adverse effect on reactivity and specificity of the product during the dating period. Labels or package inserts must include the blood group factors present on the cells, the concentration of unhemolyzed cells, the nature of any additives, and a cautionary statement that the product may show diminished reactivity during the dating period. In the case of pooled cells offered for use, there shall be no less than 30% of the cells in the pool bearing the factors stated on the labels. The usual requirements of sterility, restricted range of temperature in storage, and holding of reference samples from each lot released also apply to these products.

While the use of Reagent Red Blood Cells (Human) can be extremely helpful, their failures can also be legion. The more sophisticated blood banks will find that selection of donors regularly available to them, especially for use as screening cells, will often provide more satisfactory results than purchasing such products. The reasons for this are that cells need not be stored as long (storage deterioration in reactivity is a prime pitfall) and that the use of two separate cells is infinitely more satisfactory than even the same two cells will be in a fifty-fifty pool. Suspensions of Reagent Red Blood Cells (Human) in which only 30% of the cells in the pool carry the blood factors reduce the number of antibodies detected even further.

With panel products for identification of irregular antibodies, the solution is far less simple. Storage deterioration of reactivity is as equally severe as in the screening products, although these are not pooled, but single donor cells. To assemble a set of cells containing certain antigens in specific combinations can tax the ingenuity of anyone. The ability to market such sets on a continuing basis even with a 21-day period of dating has almost defied solution. To assure reliable results, the laboratory should have at least three different panels on automatic issue, scheduled so that one fresh panel arrives each week. Even so, not all antibodies encountered can be precisely defined with these products.

It, therefore, becomes necessary quite often to enlist the assistance of reference laboratories for solving such problems.

Sources of blood grouping reagents. Anti-A and anti-B blood grouping sera are obtained from humans immunized either through pregnancy, transfusion, or as volunteers injected with blood group specific substances.

Anti-A,B (group O) serum is also obtained from immunized human donors. Group O sera are selected according to their ability to detect very weak A antigens. Reaction against A and B cells is stronger with this reagent than with anti-A or anti-B, possibly due to a cross-reacting antibody (sometimes called anti-C). It is especially useful in detection of subgroups of A weaker than A₂. Its use is additionally recommended in routine ABO grouping as a predictable control. It should, of course, agglutinate all cells except those of group O.

Absorbed anti-A (anti-A₁) serum is prepared from serum of selected group B humans. Prior to processing, these sera contain alpha antibody (agglutinates both A₁ and A₂ cells) and alpha₁ antibody absorption with A₂ cells leaving the alpha₁ component behind. The processed serum will agglutinate A₁ and A₁B, but neither A₂ nor A₂B cells; hence, it is labeled "anti-A₁."

Red cells of A₁, A₂, B, and O specificity may be used as reagents for serum grouping tests. These may be selected within the laboratory using them, or purchased from commercial sources. Irrespective of source, working saline suspensions must be made fresh at least once each day and protected by refrigeration at 4°C when not in actual use. The group O cell will normally not be agglutinated unless some unusual antibody is present. It thus serves as a predictable control analogous to the use of group O serum in cell grouping procedures.

Another source of blood grouping reagents is a class known as Lectins. One of this group of saline extracts of seeds has anti-A specificity, and by proper--but arbitrary--adjustment, it can be made selectively to agglutinate A₁ and A₁B cells, but not red cells containing the antigen A₂. Although lima bean extracts also possess A₁ specificity, extracts of *Dolichos biflorus* are commercially available under the label "anti-A₁ Lectin." Lectins are believed to show blood group specificity by a mechanism unlike that of antigen-antibody systems containing protein. In addition, they are adjusted by dilution to sharpen their specific responses (especially *Dolichos* anti-A₁). For these reasons, Lectins may provide results that fail to agree completely when compared with absorbed anti-A sera in subgrouping specimens of group A blood.

Anti-M and anti-N sera are obtained either from humans, from immunized rabbits, or as Lectins (*Vicia graminea* with anti-N).

Anti-rhesus typing sera obtained from immunized humans are available in two general varieties. Sera containing principally incomplete or albumin-active anti-Rh₀ antibody are most common, and they are labeled "for slide or rapid tube test" along with the specific antibody they

contain. They may also have anti-Rh₀ plus anti-rh'; anti-Rh₀ plus anti-rh"; or anti-Rh₀ plus anti-rh' plus anti-rh" activity. When used according to package instructions, they will detect only the activity noted on their labels. In short supply are saline-active (or complete) anti-Rh₀ sera which are to be used only with saline-suspended erythrocytes for test tube tests. Such sera include in their label "for saline tube test only." Anti-hr', anti-hr", and the rh' variant, anti-rh^W, are also available as either "slide or rapid tube test" or "saline tube test" reagents. The need to distinguish between incomplete and complete varieties of anti-sera arises from the necessity to employ different cell suspending media, incubation periods, and glassware (slide versus test tube) in order to obtain valid results. As noted above for anti-A and anti-B grouping sera, the NIH has defined minimally acceptable titers for anti-Rh typing serum to be 32 units, for both saline and blocking type sera. Standards have also been established for avidity, a term designating the recognizable beginning of agglutination which must occur within 60 seconds. Sera must be specific for the antigens stated on the label, sterile, and free from turbidity and excessive hemoglobin content as well.

Antiserum for any blood group antigen may be obtained from humans who, lacking a given antigen on their erythrocytes, have been immunized with red cells bearing the foreign antigen.

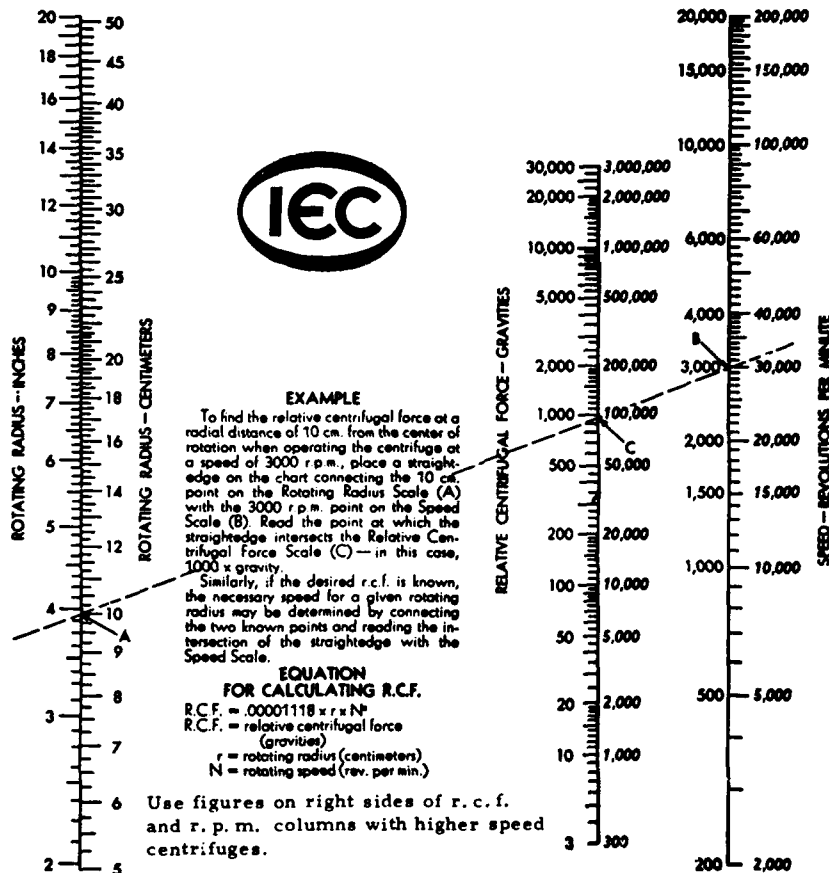
Antihuman globulin (Coombs') serum is a valuable reagent available only from a species other than man that is capable of forming an antibody against human serum protein. It is used to detect the presence of an incomplete or blocking antibody attached to, but incapable of agglutinating erythrocytes by itself. It is most often made in rabbits or goats and, just as with blood grouping sera, must also be manufactured to minimum standards defined by the NIH. Antiglobulin serum is mentioned here simply in the interest of completeness and because it is required in connection with a number of blood grouping sera, including some antigens in the Rh-Hr system. A later section is devoted entirely to pitfalls involving this reagent (see page 20).

Technique. All blood grouping reagents are packaged with a circular of instructions provided by the manufacturer. These instructions include recommendations for obtaining optimal results with the particular anti-serum. The manufacturer may find it necessary to alter the instructions periodically in order to assure accurate results, inasmuch as some change in almost any antiserum is inevitable from lot to lot. Therefore, it is necessary to read the instructions and to ascertain that no change has been introduced as new lot numbers are received. Use of recommended concentration of red cells, attention to specified proportions of cells and antiserum, care in incubation at the proper temperature for the designated time interval, and employment of proper centrifugation where required will assure accurate results for any given serum. If the package insert with a particular antiserum cautions against centrifugation, the addition of this procedure is apt to introduce a proportion of false-positive results.

Probably the least well controlled maneuver of all is centrifugation. Package instruction sheets, until very recently, uniformly stated the requirements for centrifugation in revolutions per minute (rpm) for a certain period of time. Owing to variation in the radii of centrifuge heads and speeds attained from one piece of apparatus to another, significantly wide differences in relative centrifugal force (rcf) have resulted. This variable can be standardized only when manufacturers specify the desired rcf, and laboratories operate their equipment to achieve these forces. The International Equipment Company, Needham, Massachusetts, has made available a nomograph (Fig. 1) from which rcf can be computed simply

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Figure 1.

by measuring the radius of the head and the speed of the centrifuge. The rcf is expressed as a whole number times the force of gravity, e.g., 2000 x g. All blood grouping reagents currently being purchased for the Armed Services now require the rcf values, as well as rpm values for a stated time in package circulars where centrifugation is required. Periodic calibration of centrifuges as a part of quality control within the blood bank laboratory is highly recommended.

Only the practice-acquired personal experience of a technician can minimize variables in reading results. The button of red blood cells must be resuspended carefully from the bottom of the tube. "Read as you resuspend" should be the rule! Delicate agglutinates (as in the serum grouping part of ABO testing, or evaluation of anti-Rh antibodies) can be shaken out without one ever suspecting that they had been there. With increasing experience, most technicians will indicate relative strengths of serologic reactions by assigning one to four plus symbols on their protocol sheets--which we wholeheartedly recommend--for indicating weak to complete agglutination.

Effects of certain phenomena. Rouleaux, or pseudoagglutination, is the piling up of red cells, flat surface to flat surface and under the microscope resemble stacks of coins. This may be seen in patients having altered serum proteins (M protein of multiple myeloma), or those having received plasma substitutes like dextran, polyvinylpyrrolidone (PVP), or gelatin. To the unaided eye it may resemble agglutination (and macroscopic observation may be very misleading), but the phenomenon is easily identified by microscopic examination. The addition of several drops of isotonic saline will generally disperse rouleaux, but has no effect upon true agglutination.

Factors influencing agglutination *per se*. Red blood cells must be suspended in saline rather than a high-protein medium when under test with a saline-active antiserum. To ignore the requirements of a given antiserum in this respect is to invite aberrant results.

A serum containing an incomplete antibody may be capable only of coating and not agglutinating its specific antigen. This fact can best be determined by use of the indirect antiglobulin (Coombs') technique. It is also possible that a saline antibody may be made incapable of producing crisp agglutination if the test cells are suspended in high protein or other macromolecular media.

Some blood group antigens, notably the weak variant of Rh₀, ~~Rh~~h₀, require use of the Coombs' antiglobulin technique for consistent detection. Antisera capable of detecting the antigens for Kell, Duffy, and Cellano almost always also require antiglobulin technique for agglutination to occur.

Some blood group antigens, especially Lewis, may require complement for detection. Use of heat-inactivated or long-stored serum would produce

erratic results because complement deteriorates under either of these circumstances.

Natural antibodies, especially anti-A and anti-B, are usually most reactive in the cold. When performing serum grouping tests, for example, such antibodies might fail to react at all following prolonged incubation at 37°C. In contrast, "immune" antibodies are most reactive at 37°C. Thus, reactivity of an anti-Rh antibody might be undetected following incubation either at room temperature or 4°C.

All lots of commercial sera released for distribution for which standards have been defined have been evaluated by NIH. Sera with no defined standards are also available for sale or free distribution. These generally are for blood group systems not crucial to the operation of a transfusion service and are not so well monitored by NIH. Improper storage (especially without refrigeration) promotes deterioration by loss of titer, avidity, or even specificity. The latter may be altered during storage even at the proper temperature by the slow reappearance of undesirable antibodies removed either by absorption or neutralization during the manufacturing process. Alteration in pH or electrolyte concentration may result from reconstituting a lyophilized serum differently than according to instructions; this alteration may adversely affect the ability of a serum to react properly. Contamination by saprophytic microorganisms can alter a serum by making it nonspecific.

The following comments apply to commercial typing sera. Antibodies found in random, unknown specimens also serve admirably at times as reference materials and as typing sera as well. They differ from commercially processed typing sera, however, in at least two important respects: They are almost always lower in titer, and they usually are of lower avidity. These two differences explain the delicate agglutinates and the risk of failing to detect agglutination in serum grouping tests referred to briefly on page 8 and above. Bear in mind that the same pitfalls enumerated for commercial sera may be encountered in sera you find and save for your own future use.

Hemolysis, or the rupture of red blood cells, may result from many causes. It is not infrequently associated with specific antigen-antibody reactions and is most often encountered in serum tests for ABO blood groups. Many immune isoantisera, in addition to producing agglutination, may also be capable of producing erythrocyte hemolysis. Complement, present in fresh serum, is necessary to effect *in vitro* hemolysis. The significance of hemolysis is twofold. First, it is substantive evidence that an antigen-antibody reaction has occurred, and although often overlooked, has as much significance as solid clump agglutination. Second, with reference to crossmatch tests, hemolysis **MUST** be recognized in order that a unit of blood containing the antibody producing this hemolysis will not be transfused to a recipient possessing the susceptible antigen. Hemolysis may also result from glassware having soap or chemical residue, excessive temperature of a water bath, bacterial contamination, exposure of cells to

freezing, improper salt concentration in laboratory-prepared saline, excessive age of stored cells, or from inherited chemical or metabolic abnormalities of the red cells.

Cold agglutinins are antibodies usually without blood group antigen specificity which agglutinate red cells most intensely at temperatures near 4°C. This reaction is seen not infrequently with sera of patients having primary atypical pneumonia. Because of their generally nonspecific nature, cold agglutinins may cause confusion in ABO serum grouping as well as in the major crossmatching test. Since the effect of nonspecific cold agglutinins is reversible, cells agglutinated at 4°C will, upon being warmed briefly at 37°C, disperse into a smooth suspension. Recooling the mixture causes the agglutination to reappear. It is this phenomenon that permits identification of a nonspecific cold agglutinin with confidence. Once identified, cold agglutinins can be removed from a person's serum by incubation of the serum with its own cells at 4°C or in a chipped ice bath (ca: 0-1°C) for 1 or 2 hours. It is necessary to maintain the lower temperature even during centrifugation while the serum is removed from the clot. By the same token, the nonspecific cold agglutinin may be eluted from a person's red cells by incubation of his serum at 37°C, and by maintaining this temperature when separating cells and serum. Specific antibodies in the I, M-N, P, and A-B-H systems usually are optimally active at 4°C. Almost all adults carry the I antigen on their red cells. Cells of the newborn show very little I antigen activity. In crossmatch problems due to cold agglutinins, anti-I specificity can be determined if cord cells known to be free of antigen I fail to react at 4°C.

Panagglutinins, which may be encountered in sera contaminated by bacterial growth, have the property of agglutinating all human red cells, irrespective of their antigenic composition. Panagglutinable erythrocytes may result from bacteriogenic cause, as with the Hübener-Thomsen-Friedenreich phenomenon, or from nonbacteriogenic causes as seen in acquired hemolytic anemia, which will be discussed later.

Bacteriogenic type panagglutination probably results from the action of some bacterial enzyme on the red cell surface, rendering them agglutinable by all normal adult sera; again, without regard for specific antigenic composition. Agglutination is considered to result from anti-T, an antibody which is active at room temperature and found in all normal sera except those of the newborn.

Nonbacteriogenic type panagglutinable erythrocytes are more often reactive at 37°C than at room temperature, although many specimens may fail to demonstrate this temperature differential. A somewhat more consistent point in differentiating the two is by the antihuman globulin (Coombs') reaction. Both the direct and indirect Coombs' tests are negative at 37°C in the bacteriogenic type, while these tests are usually positive in specimens of the nonbacteriogenic type.

Polyagglutinable erythrocytes differ from panagglutinable red cells in a subtle and perhaps not really fundamental way. These cells may be agglutinated by as many as 90% of normal adult sera which are ABO group-specific, but they are not agglutinated by their own serum as is the case with panagglutinable specimens. Polyagglutinability has been seen most often in patients during, or for some limited period of time following, severe viral or bacterial infection, but it has been observed in apparently healthy persons as well. Although transient in nature, the reactions occur at lower temperatures, the indirect Coombs' test is negative, and it may well be the result of *in vivo* activation of the T antigen. These specimens are free of culturable bacteria, whereas a variety of microorganisms has been recovered from panagglutinating sera.

Autoagglutinins are most often encountered in patients with acquired hemolytic anemia. They rarely can be shown to have blood group antigen specificity. Depending upon whether they are most reactive at 4° or 37°C, they may be classified as being of the cold or warm variety. Irrespective of optimal temperature, they usually are also active at room temperature. They not only interfere with blood grouping tests, but also adversely affect tests for compatibility as well. The direct antiglobulin (Coombs') test is not infrequently positive in these patients because the autoagglutinin in the serum coats the patient's own cells. The indirect antiglobulin test may be employed to demonstrate the autoagglutinin in the serum of these patients. From what has been said earlier, it can be inferred that some autoagglutinins may behave as panagglutinins also.

When blood group specificity can be shown in cases of acquired hemolytic anemia, Rh-Hr specificity may be assumed if compatibility can be demonstrated with Rh-null (---) cells. Use of elution techniques would precisely define such things as anti-hr" antibody existing in a patient possessing this antigen. It is well to realize that a host of other causes of autoimmune hemolytic anemia has been suggested. Among drugs only recently associated with such phenomena is the hypotensive agent, methyl dopa,* which appears to produce a direct antiglobulin reaction in some patients. Thus far, this drug has induced a pure gamma-type response, while others (phenacetin or p-aminosalicylic acid) have caused non-gamma-type antiglobulin reactions. Therefore, not only may these antibodies be classified according to optimal temperature but with respect to the specific kind of antiglobulin serum which can detect them as well. The so-called gamma-type antiglobulin serum is made from essentially pure gamma globulin, while the non-gamma-type serum is prepared from globulins other than gamma.

Among the causes of hemolytic anemia unrelated to the appearance of antibodies but manifested by idiosyncrasy to drugs is so-called primaquine-sensitive hemolytic anemia. These patients have an inherited deficiency of the enzyme glucose-6-phosphate dehydrogenase.

*ALDOMET®.

Irregular isoantibodies may be found in any of the blood group systems. Thus, anti-H found in the serum of A₁ and A₁B persons is unexpected (or irregular) and so is anti-A₁ in persons of A₂ or A₂B phenotypes. Unless untoward reaction to transfusion has occurred in group A or AB recipients, we ignore the subgroups of A in the operation of a transfusion service. The subgroups of A may, however, assume prime importance in serologic tests of disputed paternity, in forensic immunohematology, and in resolving discrepancies within the ABO groups when processing donor or recipient samples. Confusion from irregular anti-A₁ antibodies may be greater in samples of subgroups weaker than A₂ (A₃, A₄, etc.), particularly when such weak antigens are paired as A₂B phenotypes owing to the high probability that the A antigen may be missed completely. Such a sample could, therefore, be incorrectly classified as group B.

The presence of more than one antibody in an unknown serum often serves to demoralize the inexperienced or inadequately trained laboratory worker. Thus, an already sensitized Rh-negative patient can be expected to have an anti-Rh₀ antibody in addition to the naturally occurring ABO antibodies. If the A, B, and O cells employed for serum grouping tests contain the antigen Rh₀, for example, the unknown serum may agglutinate all three test cells if it contained saline room temperature active anti-Rh₀ in addition to anti-A and/or anti-B antibodies. It is, therefore, essential that the antigenic composition of all cells selected for serum grouping be known, and that these cells be selected in a way capable of sorting out the complex sera described.

Peculiarities in testing the blood of newborn infants. The antigens of cord blood are often much less reactive than blood from older persons. As a result, agglutinates are more easily shaken out, and false-negative direct grouping tests may be obtained. Serum grouping tests of newborn samples may not confirm cell grouping results. When a discrepancy is found in such a sample, it is necessary to know the blood group of the mother, and to remember that most, if not all, antibody in the newborn serum is of maternal origin.

False-positive cell grouping tests on cord samples may result from contamination of the specimen with Wharton's jelly. When a disproportionate number of babies appear to be group AB, it would be well to review the technique of obtaining cord blood. It is exceedingly difficult to wash such cells free of this contaminant. Collection of samples without "milking" the umbilical cord will ordinarily avoid interference from Wharton's jelly.

It occasionally happens that sufficient maternal incomplete anti-Rh₀ antibody may be present in an Rh-positive infant to fully coat the baby's cells. When such cells are tested with anti-Rh₀ sera, they may fail to be agglutinated, and thus be reported erroneously to be Rh-negative. Such an error in the laboratory may lull the clinician into a false sense of security, and compromise the chance of survival of an erythroblastotic baby. This infant's cells will invariably show a direct

positive antiglobulin test, and its true Rh designation may be extremely difficult to establish by cell grouping techniques. Elution of the coating or blocking antibody from the baby's cells and demonstration of anti-Rh₀ specificity in the eluate proves the cause of the aberrant result. In such infants, retesting after a few weeks will establish the proper Rh type for the record.

Control of reagents. The use of positive and negative control cells in blood group serology is the *sine qua non* of good procedure. Where it is possible to use the same donors over prolonged periods of time, successive lots of sera can be used with even greater confidence. It is imperative that these cells be tested for antigens of other systems in addition to those in the system for which they are selected. Thus, when screening unknown sera for irregular antibodies, the use of group O cells will avoid agglutination by ABO antibodies, and will serve to alert the technician to possible irregular agglutinins. Some useful test cells can be purchased especially for screening and particularly for the identification of antibodies. Others, such as A₁ and B, should be harvested locally, and may be from either Rh-positive or Rh-negative donors.

It is equally important to control all lots of antisera when placed in use. The need to control antiglobulin serum cannot be overemphasized. A good rule of thumb is to run both positive and negative controls once on each shift and again any time a new vial is placed into use. In general, and with all reagents, proper storage temperature and refrigeration when not actively in use is a cardinal rule to be followed. Specificity, potency, and avidity are the parameters of control that will assure accuracy of results. A system of periodic testing for these parameters will assure continuous accuracy and control of the quality of results obtained on the bench.

The antiglobulin (Coombs') test. The antiglobulin test is an exceedingly useful tool by which we can detect the presence of incomplete or blocking antibodies. The direct antiglobulin test detects *in vivo* coating or sensitization of erythrocytes. The indirect antiglobulin test detects *in vitro* sensitization. Positive tests by either technique indicate nothing more than the presence of sensitized or "coated" cells. The direct antiglobulin test is helpful in the diagnosis of erythroblastosis, autoimmune hemolytic anemias, hemolytic transfusion reactions, and the study of drug or chemical intoxications involving hemolysis. The indirect antiglobulin test, by far the most frequently used, is an essential step in the routine crossmatch test, is helpful in screening donor and recipient sera for the presence of irregular antibodies, and in the identification of antibodies so found. This test is also frequently used when studying less common blood group systems in the pursuit of genetic, anthropologic, or forensic data.

Most of the pitfalls mentioned thus far for other kinds of serum also pertain to antiglobulin reagents. Thus, proper refrigeration, protection against contamination, and continuous quality control for proof

of activity cannot be ignored. As has also been emphasized before, meticulous attention to the manufacturer's instructions for use is mandatory. While the NIH standards for its manufacture are protective to the consumer, they cannot guarantee success at the time of use.

False-positive reactions may result from:

Wharton's jelly contamination of cord samples.

Bacterial contamination of antiglobulin serum, saline, test cells, or patient's cells (as in septicemia).

Colloidal silicate or other chemical contamination of saline.

Excessive centrifugation.

Resurgence of species-specific agglutinins removed during manufacture of antiglobulin serum.

Improperly cleaned glassware.

False-negative reactions may result from:

Improper washing of test cells (carry-over of minute amounts of globulin from serum: Undue delay in completion of test may allow elution of coating antibody).

Failure to thoroughly mix the cells after each washing.

Failure to remove as much of the saline as possible after last washing before adding antiglobulin serum.

Failure to add antiglobulin serum to the test system.

Inactive antiglobulin serum (improper storage, contamination with serum-soiled droppers, etc.).

Loss or destruction of complement (serum specimens heat-inactivated or too old).

Another effect of intercurrent disease in a patient (such as septicemia causing false-positive antiglobulin test) has been found to be responsible for false-negative antiglobulin tests. As a rule, three or four saline washes will remove the residual serum and avoid neutralization of the antiglobulin serum. In multiple myeloma, however, the marked increase in globulin may prove difficult to wash out. Amounts as small as 0.0001 ml of serum seem capable of neutralizing the amount of antiglobulin serum ordinarily used in a single test. Thus, there is valid reason for the laboratory staff to be advised of the presence in the hospital of patients with certain problems which may affect the validity of laboratory tests.

The use of commercial or in-house prepared "coated" or presensitized cells as a check on antiglobulin tests regarded as negative has been proposed as an additional means of quality control. The confirmation depends on the fact that there remains in a negative test unbound antiglobulin serum which agglutinates the coated cells. It certainly does indicate that the antiglobulin serum was added to the system and that it is reactive. In very weak positive indirect antiglobulin tests, however, which may incorrectly have been regarded as negative, the coated cell confirmation test will not detect the false-negative result. More often than not, the coated cell will provide a substantial margin of confidence in the negative test, and its use can be recommended with the caution mentioned above.

Special problems. Up to this point we have been concerned with the technical problems and pitfalls which are part of a "way of life" for the blood bank laboratory bench worker. A blood bank by today's concept is an installation capable of collection, processing, and storage of human blood. But it is also more than this: A blood bank may ship its product to a remote geographic point, prepare it for infusion to a patient, process it into fractions, divert it at the end of a brief period of shelf life to a salvage program, or simply dump it down the drain. An installation which transfuses patients in addition to any of the other activities listed above is more precisely referred to as a transfusion service. Irrespective of the part or whole of the operation we may be concerned with, it is axiomatic that we strive to place the best possible product on the shelf, provide it with expert care during its period of usefulness, and get it where it ultimately may be required with unerring accuracy.

In order to insure the expertise and level of accuracy deemed necessary, comment on a few special problems may emphasize a number of pitfalls that can be avoided. What has been said thus far has generally pertained to gaining information about units of blood coming into a bank. Most of it applied equally as well to gaining similar information about potential recipients of blood. We can, therefore, quite properly recommend an acceptable crossmatching procedure, the final test for compatibility before a recipient is transfused.

EXCEPT WHERE THE DELAY MAY RESULT IN LOSS OF LIFE, THE CROSSMATCH TEST MUST BE PERFORMED BEFORE A UNIT OF BLOOD IS TRANSFUSED. A major crossmatch (recipient serum and donor cells) is required. The minor crossmatch (donor serum and recipient cells) may be omitted if all donor units and recipient samples have been screened and are thus shown to be free of irregular antibodies.

Recommended compatibility test. The major crossmatch shall include tests in saline or serum and the indirect antiglobulin (Coombs') technique. The crossmatch must be capable of detecting both natural (agglutinating) and immune (coating) antibodies. The indirect antiglobulin technique must include the use of NIH-approved antiglobulin serum in a manner recommended

by the manufacturer. Optimally one should also include albumin-serum or enzyme techniques in addition to the saline and indirect Coombs' techniques cited earlier. The average acceptable crossmatching procedure requires 1 hour.*

Use of serum not heat-inactivated and not older than 36-48 hours is mandatory to insure the presence of sufficient complement to permit antibodies dependent upon this substance to react. Because of the anti-complementary effect of many anticoagulants, plasma should not be used.

Emergency crossmatch tests. When delay in transfusion would compromise survival, the following points are to be emphasized. When ABO and Rh type are unknown, it is permissible to use group O, Rh-negative blood without crossmatch, that has been processed as follows:

1. The removal of at least 70% of the plasma from whole blood is the preferable method of reducing the levels of anti-A and anti-B.
2. When type O whole blood is to be used for other than type O recipients, it shall be free of hemolytic anti-A and anti-B. The label shall indicate the result of the test.

When this becomes necessary, the physician responsible for the case must indicate by signature the urgent nature of the case and acceptance of responsibility for this decision. As promptly as possible, the standard crossmatch is to be performed and the results of this test conveyed to the attending physician even though transfusion has been started.

When type A or B blood is used for type AB recipients, the plasma should either be removed or be free of hemolytic antibodies.

When time is sufficient that the ABO and Rh type can be determined, group-specific blood may be issued without crossmatch under conditions and provisions indicated above. It cannot be overemphasized that no substitute "short" crossmatch technique capable of protecting the recipient has yet been devised.

*

NOTE: The average acceptable crossmatching procedure requires 1 hour. This includes receipt of patient's blood specimen, blood grouping and typing, preparation of crossmatch, incubation time, performance of antiglobulin test including sufficient washing of erythrocytes, completion of transfusion request forms, entry in blood bank ledgers, and notification of ward personnel that the compatible blood is ready for issue. Whereas a period of 15-30 minutes allows for detection of a majority of antibodies that will produce destruction of erythrocytes in a patient, certain antibodies may require longer incubation periods. Examples of these are found in the Kidd and Duffy blood group systems. Our 1 hour procedure includes much more than the period of incubation and is recommended to prevent the issue of incompatible blood caused by shortcuts of serologic technique, administrative procedure, and other sources of clerical error.

Massive transfusion. Patients requiring very large volumes of blood in relatively short intervals of time (15-30 units in 4-6 hours, for example) may be expected with some degree of frequency to demonstrate stubborn oozing once the acute loss has been controlled. Somewhat analogous to the defibrination associated with *abruptio placentae* in the obstetric patient, massive bleeding in trauma patients appears to be associated with extensive soft tissue damage. When laboratory tests reveal lowered fibrinogen levels, administration of this fraction is invariably indicated. However, if fibrinogen is unavailable or its level not depressed, fresh frozen plasma (or if whole blood is indicated, units not more than a day old) may be expected to achieve hemostasis. A detailed discussion of component therapy, particularly as it affects the hereditary bleeder, is beyond the scope of this presentation.

In addition to bleeding associated with massive transfusion, an additional pitfall may be encountered if subsequent crossmatches are required for cases other than group O who have been given large volumes of group O blood. One now will be confronted with two different cell populations in the recipient sample, and although unlikely, there may be transient irregular antibody in the recipient serum. Use of group O packed red cells otherwise compatible with the recipient will introduce the least hazard with continuing need for transfusion. If the clinical situation demands whole blood, the packed red cells may be suspended in AB fresh frozen plasma screened for freedom from irregular antibodies. This maneuver will not only provide coagulation factors and restore volume, but it will also dilute the anti-A and anti-B antibody remaining with the plasma of the packed cells. To minimize the risk of contamination in processing donor blood for problems such as this, it is to be emphasized that multiple-pack, closed-system equipment must be used. It is also important to adhere to schedules of processing wherein transfusion immediately follows reconstitution of packed cell units of blood.

Multiple transfusion. In contrast to the massively transfused recipient, the multiple-transfused patient may reach an equally large volume transfused, but his need extends over a matter of several days rather than a few hours.

It is important to perform the tests for compatibility on crossmatch samples obtained fresh each day transfusion is given. This will assure the presence of complement and provide the opportunity to recognize irregular antibodies that might result from previous transfusion. Current recommendations in Standards for Blood Banks and Transfusion Services, 5th Edition, 1970 Revised, of the American Association of Blood Banks are summarized:

1. When multiple transfusions are given over a period of days, a new sample of the recipient's blood, obtained within 2 days of the next scheduled transfusion, should be used for compatibility tests to detect unexpected antibodies resulting from the previous transfusions.

2. When a patient is to receive more than one unit of blood, it is not necessary to demonstrate compatibility between the various units of blood. Pretransfusion tests for massive transfusions, including extracorporeal circulation, are adequately performed by testing each donor's serum against reagent red blood cells followed by compatibility tests with the recipient serum.

A slightly different problem is presented by the patient with aplastic anemia or other long-term need for blood. These patients may begin to have severe febrile response to each unit transfused (perhaps as a result of leuko-agglutinins) and if transfused long enough, may develop classical irregular agglutinins. Reaction resulting from leuko-agglutinins can be minimized by the use of buffy-poor blood, prepared just prior to transfusion by centrifugation and removal of the white cell mass. Vigilance in the search for the earliest appearance of irregular isoagglutinins leads to the need to identify any such antibodies that appear. Selection of donors excluding these antigens then becomes an obligation in the selection of blood for subsequent transfusion.

Two consistent, though not frequent, pitfalls involve this group of massive and/or multiple transfused patients. Crossmatch samples from heparinized recipients (most often open-heart cases) have the peculiar propensity of forming continuously recurring gel in their serum.* We can recommend no simple solution unless it is the judicious use of protamine or ionized calcium, bearing in mind that the resulting serum may be diluted or of altered electrolyte composition. The second group of patients are those on renal dialysis who are subjected to nephrectomy, transplantation, or other surgical procedures. Postoperatively these patients consistently ooze until they are given immediately fresh whole blood (not more than 1 hour old) or fresh frozen plasma. A single unit of either product may have a dramatic effect.

Exchange transfusion. The classic application of this procedure is in the treatment of erythroblastosis. Every effort must be made to crossmatch against maternal serum for this purpose because the offending antibody is in this serum and it will be of greater titer than in the infant's blood. When maternal serum cannot be obtained, the crossmatch may be performed on the baby's serum.

Additional applications of this procedure have been in drug or chemical intoxications where peritoneal lavage or renal dialysis may not be feasible, and more recently for management of hepatic coma.

Blood Components and Plasmapheresis (9)

Whole blood transfusions have been the mainstay of transfusion therapy since World War II. Yet, during World War II, the most commonly used

* Further discussion of coagulation problems found in section on consumption coagulopathy.

blood component was plasma. Though valuable for volume expansion, plasma lacked the oxygen carrying capacity of erythrocytes. Now, after two decades, the value of these as separate therapeutic agents is being realized. In fact, the majority of certified blood banks feel that a versatile program should contain not only the packed cell and plasma portions, but these should be further subdivided to provide highly potent and medically useful fractions for such problems as hemophilia and agammaglobulinemia, or for the individual needing tetanus antitoxin yet highly allergic to horse serum.

These fractions are not always part of the routine hospital blood bank operation but blood banks are the sources for the pooled plasma later used to produce these more specific portions. (Facilities such as set up at the Blood Bank Center, US Army Medical Research Laboratory, Fort Knox, Kentucky, for routine separation of the major fractions, can be similarly implemented in any certified blood bank.)

Despite the general interest in blood fractionation, there is a lack of standardization in both preparation and storage. The plasma separation operation developed at the Blood Bank Center was created to support military blood banks in the Continental United States and overseas installations, based on available equipment and particular needs in terms of pints and specific blood components, and this facility will be used to provide an example of the components prepared and how these components can be useful (10-14).

Other more complicated processing procedures are done by commercial firms and use of these specific products will be discussed briefly.

Materials and methods. The following blood components are prepared at the Blood Bank Center:

1. Fresh frozen plasma (single donor plasma (human) fresh frozen).
2. Stored liquid plasma (single donor plasma (human)) or normal human plasma (pooled).
3. Packed red cells.
4. Platelet-rich plasma (human).
5. Platelet concentrate.
6. Leukocyte-poor whole blood.
7. Leukocyte-poor red cells.
8. Small units of whole blood for pediatric use.

9. Antihemophilic globulin (Factor VIII or (AHG)-rich cryoprecipitate).

The BLOOD PACK[®] units of Fenwal Laboratories are used, including the double, triple, and quadruple pack units. Plasmapheresis units are also employed. The Sorvall RC-3 general purpose automatic refrigerated centrifuge is used in the blood component preparations. Its special features of speed in terms of revolutions per minute-relative centrifugal force (rpm-rcf), refrigeration and acceptance of a 10-15 g imbalance make component preparation possible (Fig. 2).



Figure 2.

Packed red blood cells (human).

1. The key to techniques of preparing blood components is time. Preparation follows, quite nicely, if we start with packed cells from fresh blood. The double unit pack is used to collect blood from healthy donors. After thorough cleansing of the venipuncture site, blood is collected into the larger acid-citrate-dextrose (ACD) bag with careful

attention to thorough mixing of ACD and blood. Following the collection both bags (one full, one empty) are immediately centrifuged at 4°C for 7 minutes at 5000 rpm, 6975 rcf.

2. The primary bag on the plasma expressor is suspended and the temporary closure opened to permit flow of plasma into the satellite bag. Sufficient plasma is left on the red cells to provide a 60-70% hematocrit.

3. The tubing is clamped off and sealed in two places with a dielectric sealer or metal clamps. The two bags are separated by cutting between the seals and segments of the integral donor tubing saved for pilot samples.

4. The label should carry the same information, blood group, Rh type, as for citrated whole blood (human). Red cells are stored at proper temperature (4°C); and fresh frozen plasma in satellite bag at -30°C.

Platelet-rich plasma (human). Again, the double unit packs are used in a manner similar to that for obtaining packed cells and plasma. However, the critical step here involves centrifuging the bags at 4°C at 3100 rpm (2600 rcf) for 1 minute, 35 seconds. After the centrifuge has attained a speed of 2100 rpm the brake is set at the end of the 1-minute 35-second timing. The platelet-rich plasma is obtained with the plasma expressor, the integral donor tubing dielectrically sealed, and pilot samples collected as portions of the integral tubing on either side of the seals.

When platelet concentrate is desired the platelet-rich plasma is centrifuged immediately at 4°C for 7 minutes at 5000 rpm, 6975 rcf. The plasma is then expressed into a third integral bag and enough plasma left on the bottom of platelets to allow transfer of platelets in fluid form to the patient by simple rolling of bag with contents into recipient tubing at time of infusion.

Leukocyte-poor packed blood cells or whole blood. Centrifugation is used to prepare this product from fresh blood only. While preparing leukocyte-poor blood one should take advantage of the separation products by maintaining proper criteria of time and temperature during processing.

Preparation: multiple bag unit with two satellite bags.

1. Blood is centrifuged at 4°C for 1 minute, 35 seconds after attainment of 3100 rpm (2600 rcf). The brake pedal is set.

2. The platelet- and leukocyte-rich plasma are transferred into one satellite bag.

3. The connecting tubing is clamped with a temporary clamp.

4. The unit is recentrifuged at 4°C to pack all cellular elements.
5. The temporary clamp is opened and the supernatant cell-free plasma removed.

If leukocyte-poor whole blood is desired, this plasma is returned to the primary bag. If leukocyte-poor packed cells are desired, this plasma and the plasma in the primary bag are transferred to the second satellite bag.

Plasmapheresis. The separation of plasma from whole blood by refrigerated centrifugation, and immediate return of red cells to the donor, is termed plasmapheresis. A double plasmapheresis set allows the withdrawal of two units of plasma and return of the red cells. The side arm adapter above the needle makes vein patency possible and it is maintained by slow saline drip adjusted to avoid hypervolemic diuresis. For routine donation with the equipment presently available, it is best to limit donors to a maximum of 1000 ml of plasma withdrawn per week (15,16). Following the serum protein level is essential before accepting a donor for repeated plasmapheresis; the determination should be made again after each cumulative donation of 1000 ml of plasma.

Curtailement of plasmapheresis is indicated when the total protein falls below 6.0 g/100 ml in a representative sample of the donor's serum. The total protein may not reflect the amount of depletion of gamma globulin. It is known that once depletion takes place, recovery of gamma globulin may be prolonged (12). For this reason, the electrophoretic pattern of the donor's serum proteins should be examined if total protein values are borderline (17,18).

The technique of plasmapheresis involves autotransfusion. Blood bank supervisors can appreciate necessary controls when multiple autotransfusions are carried out each day in one facility. The recommendation by Dr. Allan Kliman that group specific donors (A, B, AB, or O) be restricted to each day's plasmapheresis operation is clearly indicated to prevent blood group incompatibility in the event of clerical or technical error.

Criteria and special procedures (4).

1. The donor signs his consent after proper medical counseling.
2. A licensed physician should be present while donors are being plasmapheresed and is responsible for:
 - a. Informing donors of risks involved.
 - b. Collection and processing of blood and components.
 - c. Autotransfusion of donor's red cells within 2 hours of the bleeding.
 - d. Routine and emergency treatment of donors.
3. Laboratory and physical examination procedures should include:

a. Measurement of donor's serum protein and hematocrit or hemoglobin concentration.

b. Donor's weight recorded and compared to previous weight.

c. Measurement of donor's serum glutamic pyruvic transaminase concentration should be determined at a minimum of every 2 months.

4. Additional precautions:

a. Donation of blood shall not exceed 500 ml at any one bleeding.

b. The plasma from no more than 2 liters of blood may be retained in any one week.

c. The plasma from no more than 1 liter of blood may be retained in any 48-hour period.

d. Photographic, social security card, and other means of identification, including regional registries, should be maintained.

AHG-rich cryoprecipitates (Factor VIII component). The preparation procedure used has been the method of Dr. Judith G. Pool (19) (Figs. 3 and 4). This relatively simple method of rapidly freezing then cold thawing and separating the plasma for the remaining AHG-rich cryoprecipitate appears to be a practical technique for preparing an effective anti-hemophilic factor concentrate.



Figure 3.



Figure 4.

Discussion. Diagram 1 is used to illustrate some of the components and their usefulness.

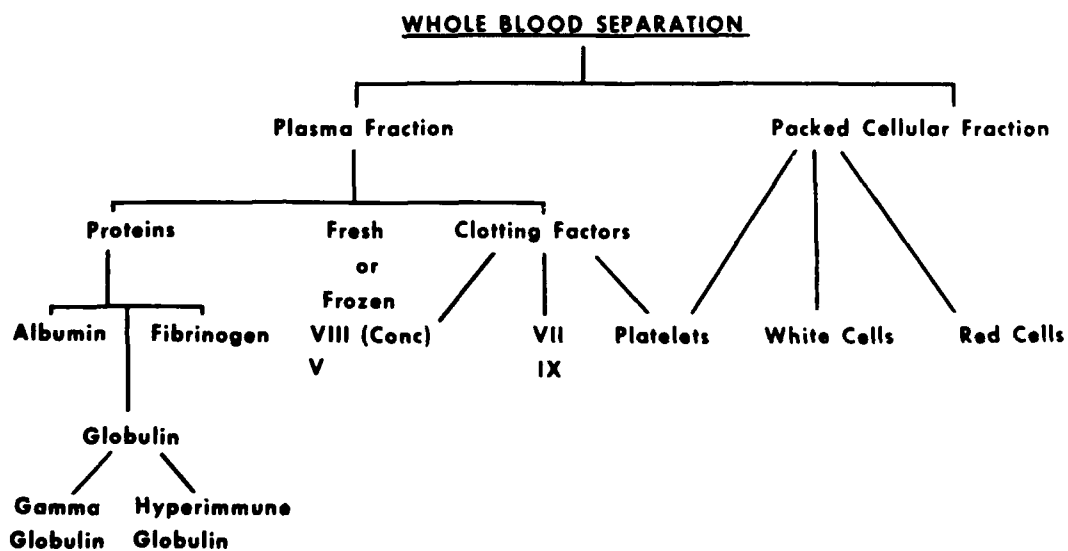


Diagram 1.

The first step of separation provides plasma as single donor, pooled or as dried products useful as a basic volume expander. Because this material has a relatively low level of antibody, it can be lifesaving in severe blood loss, as well as timesaving by not requiring crossmatching. Should these products be planned for chronic patient support rather than emergency use, single units from known donors would be preferred to reduce the risk of hepatitis.

Freshly drawn blood can provide all known clotting factors in the plasma, including platelets and, thereby, is considered the universal emergency therapy for bleeding dyscrasias. The new cryoprecipitate method of concentrating the antihemophilic factor (Factor VIII) may supply a more potent and specific component and reduce the risk of developing antibodies to this factor. This method is new and has not been fully evaluated.

In the protein separation, basic fractions consist of albumin, globulin, or fibrinogen. The albumin has value as a plasma expander, in burns, nephrotic syndrome, pancreatitis, cirrhosis, hypoproteinemia, and for its nutritional value when needed. Fibrinogen is necessary in specific deficiencies whether congenital or acquired but also carries the risk of hepatitis transmission. The globulin fraction can be used as prophylaxis for many infectious diseases, including hepatitis, and as specific replacement therapy in globulin deficient states. Additional plasma subfractions can be obtained from commercial firms and include plasmin (fibrinolysin), ceruloplasmin, plasma cholinesterase, siderophilin, and highly specific immunoglobulins protecting against measles, mumps, pertussis, or tetanus.

The critical demand for gamma globulin and other blood fractions available only from human plasma have emphasized the need for an efficient plasma salvage program. The recognition of the lability of various portions has been the stimulus for programs designed to procure fresh plasma either by the immediate separation method as described, or through plasmapheresis. Although the demand for plasma is sufficient reason to use packed red blood cells whenever possible, packed cells frequently have a distinct advantage when transfusions are primarily concerned with providing the oxygen transport function of red blood cells and not necessarily the volume function of whole blood as discussed in the monograph on genetics.

The separation process also permits adjustment of the concentration of the various components; for example, providing platelet-rich plasma or packed cells, or leukocyte-poor whole blood or red blood cells that may be therapeutically more useful in certain conditions thus possibly reducing the risk of some of the adverse reactions of whole blood transfusion.

Hemophilia and fresh plasma therapy. Fresh frozen plasma provides a means of furnishing an increase in the patient's circulating plasma. AHP is essential for the hemophiliac with soft tissue and joint bleeding, not readily controlled by local measures. When anemia and anoxia are not

critical, treatment with fresh plasma is specific. A level of at least 5-15% should be maintained, and can be attained with an initial dose of 7 ml per pound of body weight. Subsequent 3.5-5 ml doses, given at intervals of 4-12 hours for the first 48 hours, will establish control.

Concentrations between 10-35% must be achieved with surgical or dental procedures, or in cases of traumatic injury. Cryoprecipitate AHF concentrate may be very useful in avoiding excessive volume infusion in these circumstances. Achievement of effective therapy can be monitored by the laboratory with the partial thromboplastin test (13).

Platelet transfusions are clinically useful; the criteria of effectiveness are usually platelet counts and cessation of external bleeding. In the last analysis, however, the value of platelet transfusions is based largely on clinical judgment and experience (16). *In vitro* measurement of platelet functions is not a useful index of effectiveness. For example, Cronkite states, that in general, bleeding appears at higher platelet levels when thrombocytopenia develops rapidly than when the process is chronic. Thrombocytopenic bleeding may be controlled by the transfusion of viable platelets but quantitative evaluation of effectiveness is very difficult. Measurement of the lifespan of transfused platelets may be linear or exponential but the ideal method for performing such studies has not been described (20).

Freireich reports that hemorrhage resulting from thrombocytopenia in patients with acute leukemia and aplastic anemia can be controlled by platelet transfusions; severe gross hemorrhage was rarely observed when platelet counts were higher than 20,000 per cu mm. When very large doses of platelets are required in a small volume platelet concentrates (PC), prepared by centrifuging platelet-rich plasma and removing most of the plasma, are used. Platelet concentrates are 80-90% as effective as platelet-rich plasma in elevating the platelet count when prepared from plasma with a pH of 6.8 or less, achieved by the addition of citric acid (21).

Programs of Immunization and Hyperimmunization of Donors

Volunteers are required as recipients of foreign antigenic materials and subsequent to immunization as donors of serum or plasma. This requirement exists in research institutions as well as industry concerned with the production of human biologicals such as antisera.

Specific requirements (4).

1. Such programs of donor immunization should be supervised and approved by a peer review group established along the lines proposed for supervision of clinical investigations of new drugs.

2. Antigens used in such programs should, where possible, be federally licensed products.

3. If there is no suitable licensed antigen, a full description of the antigen to be used should be provided to the review group, which should be convinced of the safety of the antigen preparation and be assured that the donor will not be handicapped as a result of the procedure. All antigens should be sterile or, when viable antigens are used, should be free of all other infectious agents, as determined by appropriate test before use.

4. Schedules for administration of antigen, criteria for acceptability of plasma and results with suitable standards by the assay to be used should be made available to the review group before the procedure is begun. Any subject who responds inappropriately should be retired from the immunization program.

5. In addition to tests performed on all plasmapheresis donors, serum protein electrophoresis or quantitative immunodiffusion tests for all immunoglobulins should be performed every 4 months on donors used as a source of hyperimmune globulin preparations. Administration of antigen or plasmapheresis should be discontinued if concentrations fall below or rise significantly above the normal range established by the laboratory.

6. All records concerning the antigen, the laboratory characteristics of the plasma donor, and the immunization schedule should be retained for at least 5 years after the donor retires from the program.

7. The World Health Organization recommendations for the selection and administration of human Rh₀(D)-positive erythrocytes for stimulation of Rh-negative recipients should be followed and the following additional safeguards should be considered:

a. The cell donor's serum bilirubin (direct or total) and transaminase levels should be within normal limits, as determined within 10 days before each donation.

b. Aliquots of large quantities of freeze-preserved erythrocytes from donors whose blood is considered to carry a minimal risk of hepatitis should be used when possible.

c. The peer review group should satisfy itself that all appropriate steps have been taken to minimize the likelihood that the cells to be used as antigen will transmit hepatitis to the potential plasma donor or will result in the production of blood-group antibodies that will handicap him as a recipient of blood transfusions.

d. If immunization of sensitized plasma donors is necessary, concurrence of their personal physicians should be mandatory.

e. Sensitized women who are to be subjected to further immunization should be at least 2 years past menopause or have been permanently sterilized.

Tissue Transplantation (22)

If the evolution of tissue transplantation follows the pattern seen in routine blood transfusion therapy, then we must try to anticipate and prevent--or at least to prepare ourselves to deal with--transplantation problems that may arise in the future due to the current state of knowledge.

In a recent editorial on renal transplantation, a plea was made for avoidance of sensitization to antigens of the major histocompatibility locus (23), HL-A.

Major histocompatibility locus (HL-A). In man, many of the antigens detected serologically are under the control of a single genetic system; a single locus also controls reactivity in mixed leukocyte culture (MLC) tests. Serologic and MLC test procedures are predictive of skin-graft survival between siblings. Skin grafts between siblings identical at HL-A persist between 14 and 42 days (mean - 25 days), whereas skin grafts between most pairs dissimilar at HL-A are rejected in 7-14 days (mean - 11-12 days). A previous report from the Division of Immunology, Duke University and the Departments of Medical Genetics and Medicine, University of Wisconsin, suggested that there is a direct correlation between antigenic similarity and the failure of leukocytes to stimulate in mixed cultures. Both tests probably measure properties within the same locus--the major histocompatibility locus in man (25).

Approximately 16-32% of patients requiring renal transplantation already have detectable lymphocytotoxic antibodies at the time of transplantation (24).

Histocompatibility antigens carried on white blood cells are capable of sensitizing a recipient. Antibodies so produced can subsequently react against a donor kidney carrying the same antigens and result in very early rejection.

This problem of antigenicity along with the known ineffectiveness of white cells in the usual blood transfusion situation indicate, perhaps, that more effective ways than are currently available for separating these white cells from transfused blood should be developed. This would have a potential twofold benefit: (1) decrease the antigenic risk from an ineffective blood component, and (2) increase the availability of this component for effective blood component therapy. Kissmeyer-Nielsen et al in 1966 reported two "hyper-acute rejections" in ABO compatible grafts in

multiparous women who received multiple blood transfusions. They believed humoral antibodies to have played a part (26).

Along with the problem of white cell sensitization, one should consider the effects of sensitization following platelet transfusion, since these blood elements also carry the HL-A antigens. One editorial (27) presented a conservative estimate that 250,000 platelet transfusions are administered yearly in the United States. With the advent of more intensive therapy for neoplastic diseases and the increased use of plasmapheresis, this number can be expected to triple in the next 2 years. Available statistics indicate that a clinically important antibody will develop in 30% of patients, excluding those with leukemia and idiopathic thrombocytopenic purpura (27,28).

As a first step for dealing with individuals already sensitized and who have detectable lymphocytotoxic antibodies, a crossmatch procedure has been developed by Walford et al, 1964 (29) and Terasaki et al, 1967 (30), as modified by Stewart et al, 1969 (24). For the preoperative crossmatch the most recent sample of serum from the prospective recipient is tested against the donor's lymphocytes. The test is recorded as positive when 15% or more of the cells take up trypan blue dye used as a stain. The ability to take up dye is an indication of antigen-antibody complex formed in the cell membrane. Using this modified technique, Stewart reported immunologically successful renal allotransplantation in eight of nine patients with detectable lymphocytotoxic antibodies before operation. In each case it was possible, by means of negative crossmatch, to find a donor who carried none of the antigens against which these antibodies were directed.

Heretofore the problem of host versus graft rejection has been considered; however, in more routine blood bank practice, and in particular military blood bank practice, the universal donor problem (graft versus host reaction) is also worthy of consideration. Once again, within the framework of current practice creating future problems--organs for transplantation obviously are not as readily available as whole blood. The concept of a universal organ donor that would allow for increased flexibility of supply must be considered. Starzl has shown the importance of avoiding major blood group incompatibility in organ transplantation, but the use of an O donor to a recipient of another group might be acceptable, provided a new kind of dangerous universal donor is not created (31-34).

Rappaport et al have shown that sensitization with A and B antigens, whether on red cells or as soluble A or B substance, can elicit an accelerated skin graft rejection (35).

If A and B substance does effectively sensitize a graft recipient, then the effects of these antigenic materials on the graft donor should

be considered. This form of sensitization may be creating a situation in which the donor's lymphocytes contained within the transplanted organ may be already dangerously primed for a graft versus host rejection. In renal grafts this may not be too serious a problem, since the organ is extensively perfused before implantation. With this type of pretreatment, there is serious doubt as to whether any significant numbers of primed lymphocytes may have the opportunity to reach the host. However, when other organs such as liver, lung, pancreas, and bone marrow are considered the situation may be much more critical. It is entirely possible that under the conditions of immunosuppression required to protect the graft, small numbers of primed lymphocytes could proliferate and lead to eventual deleterious effects.

The concern of the authors with universal donor blood has led to the study of contamination of injectables with A and B substances, and to a consideration of the antigenic effect of these substances on the universal donor population. This work has shown the presence of significant amounts of A and B substances in a variety of injectables ranging from plague and influenza vaccine to placental gamma globulin (Table 4).

TABLE 4

INHIBITION STUDIES FOR A AND B BLOOD GROUP SUBSTANCES

	"A" Secretor Saliva	"B" Secretor Saliva	Plague Vaccine #1*	Plague Vaccine #2**	Placental Gamma Globulin***	Non-Placental Gamma Globulin
1:2	-	-	-		-	
1:4	-	-	-		-	
1:8	-	-	2		-	
1:16	-	-	4		-	
1:32	-	-	4		-	
1:64	-	-	4		2	
1:128	-	-	"A"	Free of Stimulating Levels of A and B Substance	3	Free of Stimulating Levels of A and B Substance
1:256	-	-	Substance		4	
1:512	-	-	40-50 µg/ml		4	
1:1024	-	-			"B"	
1:2048	2 ^s	-			Substance	
1:4096	4 ^w	-			150 µg/ml	
1:8192	4	1 ^s				
1:16,384	4	3				
	3 mg/ml	>3 mg/ml				

*Old Vaccine - discovered in August 1965.

**New Vaccine

***Recent Finding

Group O military personnel receiving multiple and repeated immunizations during their tour of duty are unsatisfactory as universal donors due to the presence of isoagglutinins and immune antibody in high titer, and the presence of hemolysin in their serum. If A and B antigens are of significance in graft rejection as Rappaport's data would indicate, and they are as ubiquitous in commonly used injectables as our findings have shown, and if the graft versus host rejection does have clinical significance--then there may already have been created potentially dangerous universal donors.

The requirement for low titer group O, universal donor, blood is adequately met by bleeding recruit donors prior to any immunizations (36-41). However, the real solution to the problem of injectables and A-B sensitization rests with newer techniques of preparation which would eliminate these antigenic contaminants.

To carry the analogy of organ transplantation and blood transfusion even further, one can consider the problems of procurement and preservation. While anticipating improved technology of organ transplantation, the problem of the most effective means of procurement can be addressed. Sadler et al describe the Uniform Anatomical Gift Act, drafted by the Commissioners on Uniform State Laws, as providing a comprehensive and modern framework for the donation of human organs for medical research, education, and therapy. Furthermore, they state that widespread adoption of the Act, which eliminates existing unnecessary legal formalities, will make available more human tissue for medical purposes (42-44).

Procurement of organs suitable for transplant will be easier when the moral, ethical, and legal problems attending the definition of death are brought into more complete agreement. As these processes interact, public acceptance, albeit gradual, can be expected to strengthen as well.

Release Notice of Scientific Treatises
in Blood Group Immunology (45)

This series of fundamental research reports from the field of blood group immunology has been prepared for the Fellowship in Blood Banking and Immunohematology for career military personnel. Some have been translated from other languages while others, originally in English, have become inaccessible. One very recent report has been included because it so perfectly supplements an older paper on the same topic. It is upon such fundamentals that the specialty of blood transfusion therapy has reached its present level of preeminence.

Introduction to the series. The translation in 1964 of Arne Gammelgaard's thesis on weak A blood resulted from our belief that it was of fundamental importance to the study of group A bloods weaker than A₂. Despite its inaccessibility for more than 20 years, this work has been cited in the references of the majority of papers dealing with this subject which have appeared in English during the past decade and more. In retrospect, much of the confusion in classification of the weak subgroups of A might have been avoided had Gammelgaard's data and conclusions been more widely appreciated. Impressed by the impact of the Gammelgaard experience, and desiring to encourage scholarly pursuit in the Fellowship in Blood Banking and Immunohematology, we have assembled 34 papers and two monographs mainly from the older scientific literature. Having thus been faced with substantial effort and expense to obtain these translations for our own use, we have been encouraged to make these papers available to workers the world over.

The papers appear in five volumes. The initial collection, Volume I, dedicated as a memorial to Ivor Dunsford, contains eight titles covering the ABO system. Volume II, containing nine papers, covers the secretion of blood group specific substances and the Lewis system. Volume III contains four papers dealing with the MN and P systems and a monograph by Ludwig Hirszfeld. Volume IV contains six papers and a monograph by Fritz Schiff covering anthropologic and other applications of blood grouping data. Volume V commemorates the centennial of the birth of Karl Landsteiner. Six of the seven papers in this volume are by Landsteiner and deal with basic concepts in immunology.

It is hoped that the series of papers will stimulate established workers in the field of immunohematology and provide newcomers to this area a sound indoctrination in this exciting discipline.

Volume I. ABO System-Dunsford Memorial

Landsteiner, K.: Contribution to the knowledge of the antienzymatic, lytic and agglutinating effects of blood serum and lymph. (Zur Kenntnis der antifermentativen, lytischen und agglutinierenden Wirkungen des Blutserums und der Lymphe.) Centr. Bakt. Orig. 27: 357-366, 1900.

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Volume II. Secretion of Blood Group Substances and Lewis System

- Schiff, F.: A contribution toward the knowledge of blood-group-specific antigens and antibodies. (Zur Kenntnis blutgruppenspezifischer Antigene und Antikörper.) Klin. Woch. 1:679-680, 1924.
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Bernstein, F.: Continued investigations in the theory of blood groups. (Fortgesetzte Untersuchungen aus der Theorie der Blutgruppen.) Zeitschrift für Induktive Abstammungs- und Vererbungslehre, 56(2): 233-273, 1930.

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Consumption Coagulopathy (45a)

One of the most important recent contributions to clinical medicine is the unifying concept of disseminated intravascular coagulation (DIC) or consumption coagulopathy (CC) as an intermediary mechanism of disease and death (46,47). Recognition of CC as a clinical entity involving the coagulability of blood was related to the discovery by Schneider (48) that the active principle of placental toxin in *placenta abruptio* was thromboplastin. The nature of the blood coagulation mechanism and disseminated intravascular coagulation to problems in obstetrics and gynecology was reported by Seegers and Schneider as early as 1951 (49). CC has great clinical importance, not only because it is a common pathway of death in diverse multiple diseases and disorders, but also because with proper and precise treatment an extended interval of life can be achieved, allowing additional time for diagnosis and treatment of the primary disease. At such a time the CC will subside spontaneously. In some cases, the underlying disease, e.g., malignancy, is incurable and prognosis for extended life is hopeless. CC is recognized in a growing list of conditions (Table 5) and often is amenable to treatment. It must be appreciated that not all diseases manifest CC, nor do those conditions in which a mild degree of CC may be present require treatment. Unquestionably, many cases of CC are subclinical, undetected, and spontaneously remit. But in those cases where it is an imminent threat to life, accurate diagnosis and prompt, correct therapy are required if the patient is to recover.

As proposed by Seegers (50) the coagulation mechanism involves three basic reactions: (1) the formation of autoprothrombin C (F-X_a, thrombo-kinase, Stuart factor); (2) the formation of thrombin; and (3) the formation of fibrin. An updated version of these concepts is presented in Figure 5.

CC is a distortion of normal coagulation and fibrinolytic mechanisms. Among coagulationists, there is a growing view that under normal conditions, there is a nicely balanced, dynamic equilibrium between continuous coagulation by the intrinsic coagulation system in the arteries and arterioles, and continuous fibrinolysis by the fibrinolytic system primarily in the capillaries. The schematic in Figure 5 summarizes the events discussed in this monograph and should be referred to frequently.

TABLE 5

CLINICAL ENTITIES ASSOCIATED WITH CONSUMPTION COAGULOPATHIES*

I. ACTIVATION OF CLOTTING SYSTEM PREDOMINANTLY:	
CLINICAL ENTITY	
1. HUMAN EQUIVALENT OF SANARELLI-SCHWARTZMAN REACTION	
2. THROMBOTIC THROMBOCYTOPENIC PURPURA (MOSCHOWITZ' SYNDROME)	
3. PURPURA FULMINANS	
4. HEMOLYTIC-UREMIC SYNDROME (GASSER'S SYNDROME)	
5. KASABACH-MERRITT SYNDROME	
6. SEPTIC SHOCK	
7. INCOMPATIBLE HEMOLYTIC BLOOD TRANSFUSION DISEASE	
II. ACTIVATION OF FIBRINOLYTIC SYSTEM PREDOMINANTLY:	
CLINICAL ENTITY	
1. PRE-OPERATIVE ANXIETY	
2. PULMONARY SURGERY	
3. PROSTATE GLAND SURGERY	
4. NEUROSURGERY	
5. OPEN HEART SURGERY	
III. SIMULTANEOUS OR CO-EQUAL ACTIVATION OF CLOTTING SYSTEM AND FIBRINOLYTIC SYSTEM:	
CLINICAL ENTITY	
1. ABRUPTIO PLACENTA	
2. DEAD FETUS SYNDROME	
3. AMNIOTIC FLUID EMBOLISM	
IV. OTHER CLINICAL ENTITIES EXHIBITING CONSUMPTION COAGULOPATHY:	
FULMINANT FAT EMBOLISM SYNDROME	
ACUTE PROMYELOCYTIC LEUKEMIA	
THROMBOTIC THROMBOCYTOPENIC PURPURA	
PAROXYSMAL NOCTURNAL HEMOGLOBINURIA	
SICKLE CELL DISEASE	
FAVISM	
PAROXYSMAL COLD HEMOGLOBINURIA	
MALARIA	
PARATHORMONE POISONING	
VIRUS AND RICKETTSIAL DISEASES	
DIETARY LIPIDS	
NON-BACTERIAL THROMBOTIC ENDOCARDITIS	
ACUTE RENAL FAILURE	
"HYPER-IMMUNE" RESPONSE, KIDNEY ALLOGRAFT	
THROMBOCYTOPENIA (SOME CASES)	
CRYOPRINEMIA	
PRIMARY PULMONARY HYPERTENSION	
ECLAMPSIA	
SEPTIC ABORTION	
PREMATURE RUPTURE OF MEMBRANES WITH CHORIO-AMNIONITIS	
HYDATIDIFORM MOLE	
PANHYPOPLUTITARISM	
CARCINOMA	
HEMORRHAGIC PANCREATITIS	
ULCERS, GASTROINTESTINAL TRACT	
PSEUDOMEMBRANOUS ENTEROCOLITIS	
CYANOTIC CONGENITAL HEART DISEASE	
INFANTILE DIARRHEA	
MENINGOCOCCEMIA	
WATERHOUSE-FRIDERICHSEN SYNDROME	
INTUSSUSCEPTION	
PYODERMA GANGRENOSUM	
GENERALIZED VACCINIA	
MILIARY TUBERCULOSIS	
UNDISCOVERED CONDITIONS	

*IN THE CLINICAL COURSE OF CONSUMPTION COAGULOPATHY THE BASIC PATHOPHYSIOLOGY MAY CHANGE FROM ONE CATEGORY TO ANOTHER, REQUIRING PERIODIC MONITORING OF THE DISORDER BY A "COAGULATION PANEL" OF TESTS.

This table lists 50 clinical entities in which consumption coagulopathy has been associated in the role of an intermediary mechanism of the disease and death. Although not absolutely consistent in every clinical case, several conditions can be typically characterized as resulting from: (1) primary activation of the coagulation system; (2) primary activation of the fibrinolytic system; or (3) simultaneous or equal activation of both the clotting system and the fibrinolytic system. Since consumption coagulopathy is a dynamic pathophysiologic disorder, being further modulated by the clinical events and evolution of the underlying and triggering disease or disorder, it becomes apparent that the type of consumption coagulopathy may change character over an interval of time. Modified after Mammen and used with permission.

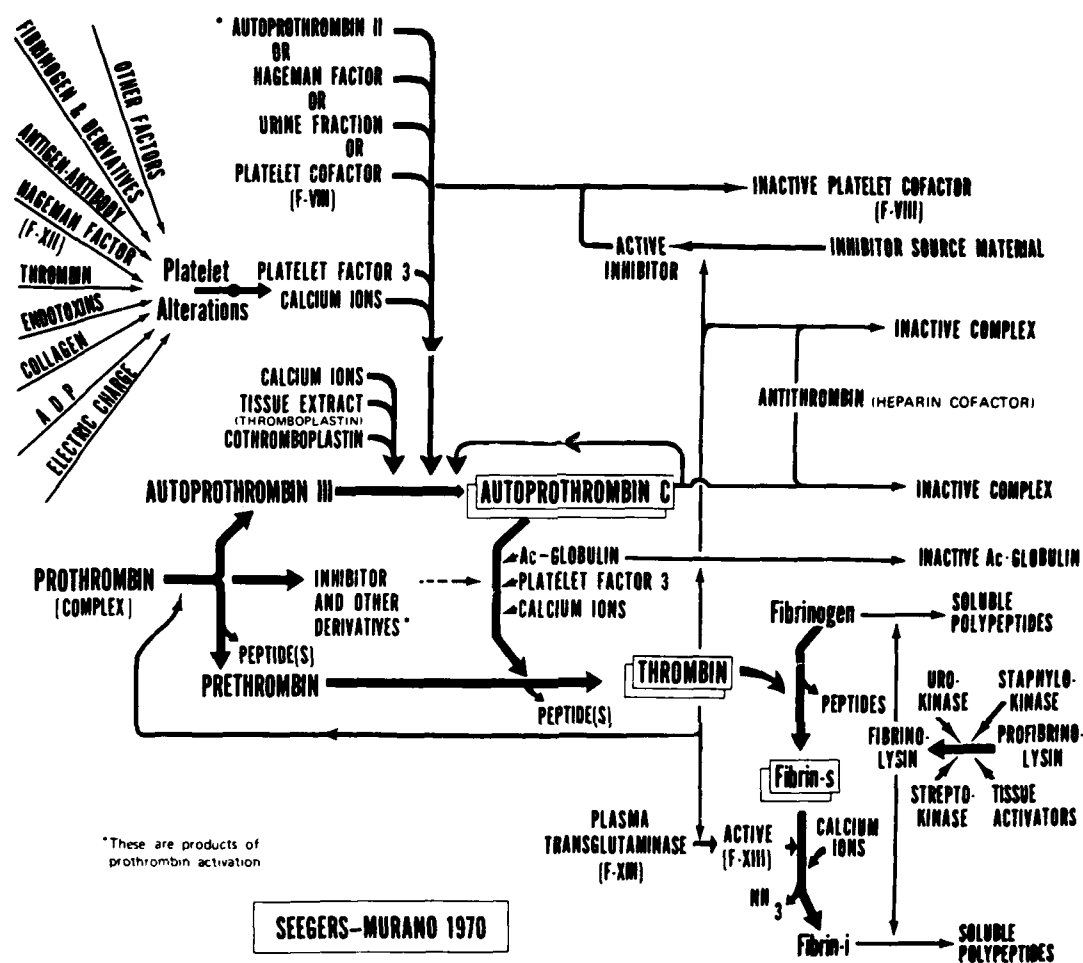


Figure 5. The recent scheme by Seegers and Murano indicates that the precoagulants generated during the activation of clotting are neutralized by inhibitors and that fibrin can be lysed by the fibrinolytic mechanism and cellular phagocytosis. Heparin accelerates the neutralization of thrombin and autoprothrombin C by antithrombin. Heparin also enhances the anticoagulant properties of FSP and functions synergistically with antithrombin in consumption coagulopathy produced by primary activation of the fibrinolytic system. Trasylol inhibits fibrinolytic activity, fibrinolysin activators, and, in addition, inhibits thromboplastin in the extrinsic clotting system. Epsilon aminocaproic acid inhibits only the action of fibrinolytic activators.

Blood is continuously clotted and lysed intravascularly at a slow rate. In a substantial variety of diseases the two basic counterpoised activities of clotting and lysis can be accelerated, either singly or concomitantly. Thus, there are three types of CC. It is important to diagnose and categorize each type correctly, because treatment is type-specific and can be lethal if incorrectly utilized. The three major types of CC are: (1) primary or predominant activation of the intrinsic and/or extrinsic coagulation system with a beneficial, mild secondary activation of the fibrinolytic system; (2) primary or predominant activation of the fibrinolytic system; (3) activation of both systems simultaneously. While the classification of clinical entities associated with CC (Table 5) is helpful, it is not to be considered as an absolute guide, since other concomitant factors in the clinical context may modulate the CC sufficiently to alter its character and, therefore, the therapy required. Thus, the clinical course of CC must be monitored frequently by appropriate, simple laboratory methods.

Intrinsic and extrinsic coagulation systems. Figure 6 summarizes the significant differences between the intrinsic and extrinsic systems.

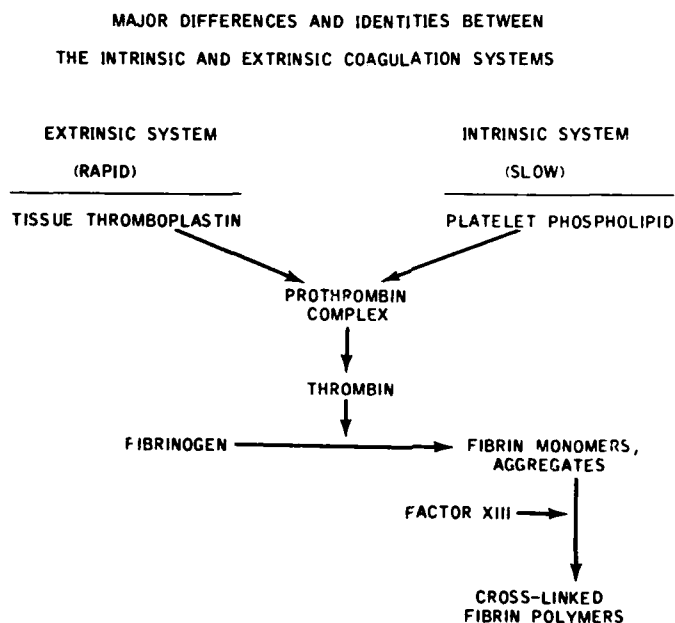


Figure 6. The major differences and the major identities between the intrinsic and the extrinsic coagulation systems are summarized in this figure. Note that the extrinsic system of coagulation is more rapid, clotting plasma in minutes, and requires the action of platelet phospholipids. From the point where prothrombin complexes are formed, both coagulation systems have a common route.

The extrinsic coagulation system is accelerated by the presence of tissue thromboplastin and results in a rapid clotting of the plasma within seconds. The intrinsic coagulation system is activated by the presence of platelet phospholipid and is slower, requiring minutes for the coagulation of plasma. From that point onward in the coagulation sequence where prothrombin complex is converted to thrombin, the intrinsic and extrinsic coagulation systems follow a common pathway (Figs. 5 and 6). For purposes of simplicity, we will speak in terms of the intrinsic coagulation system throughout this monograph, with the understanding that the extrinsic coagulation system is also included, but is less frequently involved in cases of CC.

Primary activation of the intrinsic coagulation system. The significant events in the primary activation of the intrinsic coagulation system are summarized in Figure 7. Note that with the activation of the intrinsic coagulation system predominantly there is an intravascular conversion of plasma to serum with the production of disseminated minute fibrin clots. If this course of events were allowed to continue with the transformation of plasma from a liquid to a solid phase, death would obviously ensue. The fact that patients do survive the catastrophe is explained by the secondary activation of the fibrinolytic system as shown in Figure 7.

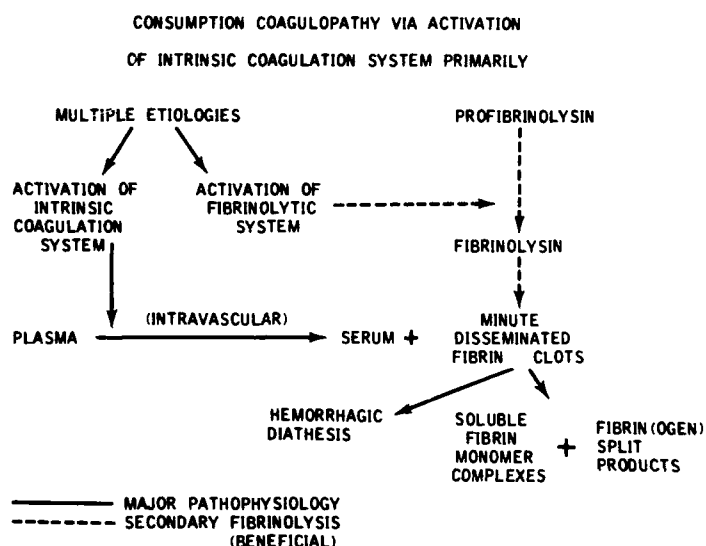


Figure 7. This figure summarizes the significant metabolic events in the primary activation of the intrinsic coagulation system. Note that there is a conversion intravascularly of plasma to serum with the formation of minute disseminated fibrin clots, soluble fibrin monomer complexes, and fibrinogen split products. Also note that there is a secondary, subordinate lifesaving activation of the fibrinolytic system which is NOT to be confused with primary activation of the fibrinolytic system. (See text.)

The basic pathophysiology of primary activation of the intrinsic coagulation system is summarized in Figure 8. With the intravascular conversion of plasma into serum, alterations occur among several coagulation factors. The differences in these factors between plasma levels under normal conditions and under pathological conditions are indicated. At this point attention is directed to the presence of soluble fibrin monomer complexes (SFMC) which appear with the activation of the intrinsic coagulation system. Among the SFMC is a uniquely clottable SFMC. The pivotal diagnostic significance of this clottable polypeptide will be discussed subsequently.

BASIC PATHOPHYSIOLOGY OF PRIMARY ACTIVATION
OF INTRINSIC COAGULATION SYSTEM

PLASMA INTRAVASCULAR SERUM

PLASMA LEVEL	FACTOR NUMBER	FACTOR NAME	SERUM LEVEL
NORMAL VALUES	I	FIBRINOGEN	↓
	II	PROTHROMBIN	↓
	III	TISSUE THROMBOPLASTIN	
	IV	CALCIUM	
	V	ACCELERATOR GLOBULIN	↓
	VI	NONE	
	VII	AUTOPROTHROMBIN - I (PROCONVERTIN)	
	VIII	ANTHEMOPHILIC GLOBULIN	↓
	IX	AUTOPROTHROMBIN - II (PTC) (CHRISTMAS FACTOR)	CONSUMED
	X	AUTOPROTHROMBIN - III - C (STUART-PROWER FACTOR)	CONSUMED
	XI	PTA	
	XII	HAGEMAN FACTOR	
	XIII	FIBRIN STABILIZING FACTOR	CONSUMED
		PLATELET COUNT	REDUCED
		SOLUBLE FIBRIN MONOMER COMPLEXES	PRESENT
		FIBRINOGEN SPLIT PRODUCTS	PRESENT

Figure 8. This figure summarizes the basic pathophysiology of the primary activation of the intrinsic coagulation system. Note that there is an intravascular conversion of plasma to serum. Those coagulation factors which are significantly deviated from normal levels are shown on the extreme right. Those changes particularly to be noted are the drop in the fibrinogen level, the prothrombin activity, and the platelet count; all occurring as plasma is converted to serum. Note, also, that there are formed soluble fibrin monomer complexes (including the clottable form) and fibrinogen split products. (See text.)

Primary activation of the fibrinolytic system. The important events in the primary activation of the fibrinolytic system are schematically shown in Figure 9. Note that profibrinolysin activators, rather than fibrinolysin *per se*, control the rate of fibrinolytic activity. Furthermore, it should be appreciated that fibrinolysin acts on the substrate, the fibrinogen of plasma, producing a mimetic "serum." This "serum" is analogous to, but not identical with, the true serum produced by the primary activation of the intrinsic coagulation system. The differences between these two types of "sera" can be easily understood by referring to Figure 6. With primary activation of the intrinsic coagulation system, it will be noted that prothrombin and thrombin are involved in the sequence of clotting events. On the other hand, with primary activation of the fibrinolytic system, fibrinogen is utilized directly as a substrate for fibrinolysin, independently of the events leading to thrombin formation. Thus, because different enzyme-substrate systems are involved, it follows that "sera" produced by primary activation of each of these two systems will necessarily be characteristically and diagnostically different.

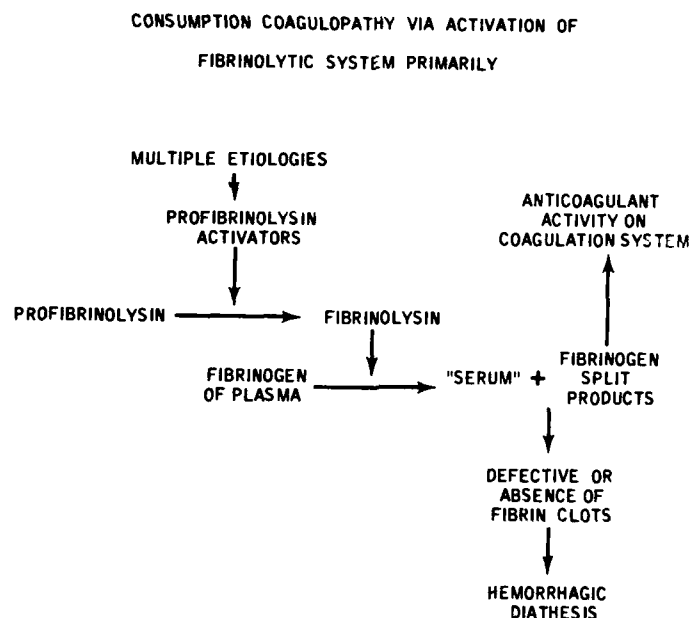


Figure 9. The important metabolic events in the primary activation of the fibrinolytic system are shown in this figure. Note that profibrinolysin activators rather than fibrinolysin control the rate of activity on the substrate, the fibrinogen of plasma, and its conversion into fibrinogen split products. A study of Figure 6 will reveal that primary activation of the coagulation system occurs at a different point in the metabolic map than does primary activation of the fibrinolytic system, while a "serum" with demonstrable distinctions from true serum is formed by primary activation of the fibrinolytic system. (See text.) Compare Figures 8 and 10.

In Figure 9 it will be seen that coincidental with the production of "serum," when the fibrinolytic system is primarily activated, fibrinogen split products (FSP) are produced as well. These FSP consist of a heterogeneous collection of polypeptides (which the coagulationists are now characterizing chemically) and have a profound anticoagulant effect on the coagulation system. Under pathological conditions FSP can exacerbate the hemorrhagic diathesis. Furthermore, any clots produced in the presence of this activated system are likely to be defective. The absence of clot formation is more often the case.

Figure 10 summarizes the coagulation events typical, or characteristic of, primary activation of the fibrinolytic system. Resultant changes among the several coagulation factors are indicated as again plasma is intravascularly converted to a "serum." A comparison of the resultant "sera" produced by both primary systems (compare Figs. 8 and 10) will be most instructive. It should be evident then that, while activation of both the fibrinolytic system and the coagulation system produces "sera," these two "sera" have diagnostically different characteristics and thus form the basis for a differential diagnosis.

BASIC PATHOPHYSIOLOGY OF PRIMARY ACTIVATION
OF FIBRINOLYTIC SYSTEM

PLASMA		INTRAVASCULAR		HEMATIC SERUM	
PLASMA LEVEL	FACTOR NUMBER	FACTOR NAME		SERUM LEVEL	
NORMAL VALUES	I	FIBRINOGEN		↓	
	II	PROTHROMBIN			NORMAL
	III	TISSUE THROMBOPLASTIN			
	IV	CALCIUM			
	V	ACCELERATOR GLOBULIN		↓	
	VI	NONE			
	VII	AUTOPROTHROMBIN - I (PROCONVERTIN)			
	VIII	ANTHEMOPLHIC GLOBULIN		↓	
	IX	AUTOPROTHROMBIN - II (PTC) CHRISTMAS FACTOR			
	X	AUTOPROTHROMBIN - III (STUART PROWER FACTOR)			
	XI	PTA			
	XII	HAGEMAN FACTOR			
	XIII	FIBRIN STABILIZING FACTOR			
		PLATELET COUNT		NORMAL USUALLY	
		CLOTTABLE SOLUBLE FIBRIN MONOMER COMPLEX		ABSENT	
		FIBRINOGEN SPLIT PRODUCTS		↑ ↑ ↑	

Figure 10. This figure summarizes the basic pathophysiology of the primary activation of the fibrinolytic system as plasma is converted intravascularly to a "serum." Those coagulation factors which are significantly deviated from normal levels are shown in the extreme right. Note that as "serum" is formed there is a drop in the level of fibrinogen. In contrast to the primary activation of the coagulation system, note that in this figure the prothrombin activity remains normal and the platelet count usually remains normal. Note especially that clottable SFMC is not produced in this type of CC. (See text.) However, fibrinogen split products are markedly increased.

Differential diagnosis of consumption coagulopathy. In Table 6 are listed some of the coagulation parameters altered in the course of CC, both with primary activation of the intrinsic coagulation system and with primary activation of the fibrinolytic system. It will be seen that fibrinogen, and Factor V and Factor VIII activity, are depressed in both conditions. The features of differential importance, then, include platelets, the presence or absence of the SFMC, and the degree of fibrinolytic activity. Based on these parameters, a coagulation profile or panel composed of simple tests can be defined which will lend itself to convenient periodic repetition.

TABLE 6*

PARAMETERS	ACTIVATION OF INTRINSIC COAGULATION SYSTEM PRIMARILY (COMMON)	ACTIVATION OF FIBRINOLYTIC SYSTEM PRIMARILY (RARE)	CO-EQUAL ACTIVATION OF BOTH SYSTEMS (FREQUENT)
FIBRINOGEN	↓	↓	↓
PLATELETS	↓	NORMAL (ALMOST ALWAYS)	↓
FACTORS V, VIII ACTIVITIES	↓	↓	↓
PROTHROMBIN TIME	PROLONGED	NORMAL TO PROLONGED	PROLONGED
CLOTTABLE SOLUBLE FIBRIN MONOMER COMPLEX	PRESENT	ABSENT	PROBABLY NEGATIVE
FIBRINOLYTIC ACTIVITY	NORMAL TO SLIGHTLY ↑	↑ TO ↑ ↑ ↑	↑ ↑ ↑

* This table lists some of the coagulation parameters altered in the course of consumption coagulopathy. In one column the changes associated with the primary activation of the intrinsic coagulation system are listed. In the other column changes associated with the primary activation of the fibrinolytic system are listed. Note that the most useful parameters diagnostically consist of the fibrinogen level, the platelet count, the prothrombin time, the absence or presence of the clottable soluble fibrin monomer complex, and the determination of fibrinolytic activity. It becomes apparent that determination of Factor V and Factor VIII activities is neither essential nor of differential diagnostic value. With equal activation of both systems a combination of qualitative changes in the parameters will be evident.

Consumption coagulopathy panel. In Table 7 are listed in one column the parameters of coagulation which are significantly affected in the disorders of CC, and in the other column appropriate corresponding tests which constitute the CC panel used by us. This panel is repeated as frequently as is necessary, or at least at 12-hour intervals. By observing the values of the several coagulation factors as they shift toward serum or toward plasma, the clinician may evaluate effectiveness of treatment and adjust dosage. Patients must also be observed for a possible change

in the type of CC as a consequence of the clinical evolution of the underlying disease. This constitutes another reason for periodic repetition of the CC panel. Since these tests will be repeated frequently, they must be simple, reliable, and the panel must consist of a minimum set of tests without sacrificing accuracy of diagnosis.

TABLE 7*

PARAMETER AFFECTED	APPROPRIATE TEST
FIBRINOGEN	"PLASMA" FIBRINOGEN LEVEL
PLATELETS	PLATELET COUNT
PROTHROMBIN	PROTHROMBIN TIME
CLOTTABLE SOLUBLE FIBRIN MONOMER COMPLEX	GLUECK MODIFICATION OF ETHANOL GELATION TEST OF BREEN AND TULLIS
PROFIBRINOLYSIN ACTIVATORS	EUGLOBULIN LYSIS TIME
ACTIVITY OF INTRINSIC SYSTEM*	PARTIAL THROMBOPLASTIN TIME
ERYTHROCYTES	FRAGMENTATION NOTED IN PERIPHERAL BLOOD SMEAR

*Not to be repeated while the patient is being treated with heparin.

* In this table we have listed the coagulation parameter and the corresponding simple, appropriate tests which constitute our "consumption coagulopathy panel." These parameters are measured by the indicated tests periodically, at least every 12 hours, and more often if indicated. The quantitative variation in these periodic determinations among the several parameters is an index of the efficacy of treatment, the adequacy of dosage levels, and a survey of the character of the CC.

While the determination of the parameters listed in the left hand column is all that is necessary to diagnose and evaluate CC, it is fully expected that the list of appropriate tests in the right hand column will vary from hospital laboratory to hospital laboratory, reflecting aspects of interest, sophistication, and capability. The basic set of coagulation parameters will be the same regardless of which type of test is used for evaluation.

In Table 7, we have listed those tests which we have found to be reliable in our laboratories. Other laboratories undoubtedly will prefer their own particular constellation of tests. Because there is a multiplicity of coagulation tests and because there are differences of interest and of capability from laboratory to laboratory, it will probably be necessary for each laboratory to assemble its own CC panel. Thus, the clinician suspecting CC must, from the very beginning, work in close consultation with his pathologist in the diagnosis and management of CC.

While most of the tests in Table 7 are well known to all laboratories, brief comment about some of them is required.

With primary activation of the coagulation system, platelets will be significantly depressed almost invariably. On the other hand, platelets are usually at normal levels in presence of primary activation of the fibrinolytic system. In addition, platelet counts vary directly with the amount of blood given. Thus, platelet counts are almost always, but not absolutely, reliable in the differential diagnosis.

It is not essential to assay the activity of Factors V and VIII since both are depressed in either condition. In addition, the assay methods are not generally available in most hospital laboratories. For these reasons Factor V and Factor VIII activity assays have not been included in our consumption coagulopathy panel.

The two-stage prothrombin time is a more accurate measure of prothrombin activity, but the one-stage Quick prothrombin time has proven useful in application to clinical problems. It should be noted, however, that prothrombin time may be prolonged in both primary types of CC. In activation of intrinsic coagulation system primarily prothrombin is consumed. In primary fibrinolysis the FSP act as inhibitors of prothrombin activation rendering the assay procedure dubious.

The Glueck modification (52) of the Ethanol Gelation Test (53,54) is most useful in the diagnosis of the type of CC and is of pivotal importance. This test will detect the presence or absence of a clottable soluble fibrin monomer complex (SFMC) in plasma (52-55). According to Niewiarowski (56) there are at least two forms of SFMC. One form, the direct result of the action of thrombin, consists of a complex of fibrinogen and fibrin monomers and is clottable by thrombin. The other form of SFMC is nonclottable and consists of soluble fibrin monomers complexed with fibrinogen split products. The Ethanol Gelation Test detects the presence of the clottable form of SFMC. Under the conditions of the Glueck modification (52) a positive test* indicates acceleration of the activity of the

*We have noted the occurrence of false positive tests in the presence of some dysproteinemias and hyperglobulinemias which can be recognized by the addition of 1 drop of 0.1 N NaOH as the last step in the Glueck modification. False positive "gels" disappear and true gels persist in the alkalinized test system. At the suggestion of Dr. Glueck we routinely add to the working solution of the Ethanol Gelation Test 2 mg/ml of soybean trypsin inhibitor to arrest the enzymatic destruction of clottable SFMC by fibrinolysin.

coagulation system. In cases of CC due to primary activation of the fibrinolytic system, the Ethanol Gelation Test will be negative, since the clottable form of SFMC will not be present. This test is highly pertinent, since it discriminates between accelerated intrinsic coagulation and accelerated fibrinolysis.

The euglobulin lysis time is a test of considerable differential significance also, since it measures the activity of profibrinolysin activators controlling the rate of fibrinolysis (56). It can be anticipated from a study of Figure 7 that the euglobulin lysis time will be normal or perhaps slightly elevated in cases of primary activation of the intrinsic coagulation system. Under pathologic conditions with primary activation of the fibrinolytic system (Fig. 9), the euglobulin lysis time is usually markedly shortened, often to a matter of a few minutes. In rare, more severe cases, activation of this system may be so marked, and the fibrinogen level so depressed, that no clot can be formed initially in the performance of the euglobulin lysis time test.

The partial thromboplastin time is a measure of the overall activity of the intrinsic coagulation system. This test is diagnostically useful initially only before treatment. In those cases of consumption coagulopathy treated with heparin (see below), the partial thromboplastin time will be markedly prolonged due to the heparin. It is pointless to repeat the test after the diagnosis has been made initially.

Finally, CC sometimes--but not always--is accompanied by fragmentation of erythrocytes (58,59), a diagnostic feature easily detected by examination of a finger-stick preparation of a peripheral blood smear.

Principles of treatment. Treatment will be dictated by the determination of the type of CC present: (1) primary activation of the intrinsic coagulation system; (2) primary activation of the fibrinolytic system; or (3) equal activation of both systems. Approximately 90% or more of cases of CC are due to primary activation of the intrinsic coagulation system (60). CC cases due to primary activation of the fibrinolytic system are encountered rarely.

The basic principle of treatment of CC is the intravascular reversion of serum or mimetic "serum" to plasma by the titration of the patient with continuous intravenous administration of the appropriate drug in dosage regulated by the response of individual coagulation parameters in a periodically repeated standard CC panel (Fig. 11). As the coagulation parameters revert to normal under this management, and as the underlying disease triggering the CC has been brought under control, therapy for CC can be quickly discontinued since the CC will then spontaneously remit.

Primary activation of the intrinsic coagulation system produces a CC which is treated only with intravenous heparin continuously administered. Initially, 5,000-15,000 units of heparin may be given intravenously;

**BASIC PRINCIPLE OF TREATMENT
OF CONSUMPTION COAGULOPATHY**

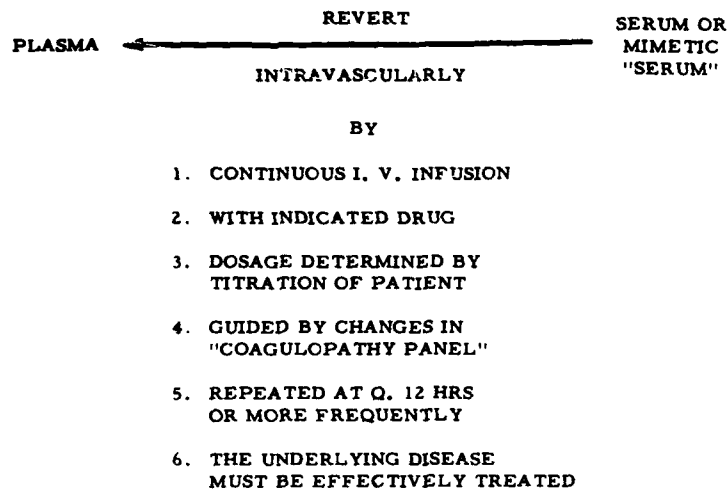


Figure 11. This figure summarizes the important aspects of the basic principle of the treatment of consumption coagulopathy, essentially the intravascular conversion of serum back to plasma. (See text for details.)

thereafter 10,000-30,000 units of heparin per 24 hours are administered as guided by changes in the CC panel. Consumption coagulopathies of the type resulting from primary activation of the fibrinolytic system require aminocaproic acid* given intravenously; 4-5 g (16-20 ml) should be administered by infusion during the first hour of treatment followed by a continuing infusion at the rate of 1 g (4 ml) per hour. This regimen would ordinarily be continued for about 8 hours or until the bleeding situation has been controlled. Aminocaproic acid inhibits only the action of the fibrinolysin activators--not fibrinolysin itself--producing a therapeutic lag. In CC, with activation of both the coagulation system and fibrinolytic system, it is best to use heparin first with aminocaproic acid added subsequently if the laboratory data of repeated consumption coagulopathy panels indicate the need. Trasylol at appropriate dosage levels (known to inhibit fibrinolytic activity, fibrinolysin activators, and thromboplastic activity) would be an appropriate drug for the treatment of CC of the equal activation type (61). However, Trasylol has not been released for clinical use by the Federal Drug Administration.

It is of crucial importance to categorize CC properly, and to select the appropriate treatment (60). Erroneous interchanging of therapy relative to the category of CC present can be lethal. For example, if CC due

*AMICAR - Lederle.

to primary activation of the coagulation system is incorrectly treated with aminocaproic acid, it will inhibit the lifesaving activity of the secondarily activated fibrinolytic system which assures the removal of fibrin as it is formed. On the other hand, the use of heparin in a case of CC of the type produced by primary activation of the fibrinolytic system will merely enhance the anticoagulant properties of the fibrinogen-split products, function synergistically with antithrombin, and exacerbate the hemorrhagic diathesis.

The appropriate drug must not only be administered in sufficient dosage to produce the desired effect; it must be given continuously. Otherwise, there will be intervals as the drug concentration falls below efficacious levels, which will permit incremental exacerbations of the CC. To avoid these intermittent therapeutic lapses, the selected drug, whether heparin or aminocaproic acid, should be administered intravenously and continuously by the use of infusion pumps.

To reiterate, as soon as the underlying disease triggering the CC is effectively treated, heparin therapy can be discontinued immediately. The heparin effect will be dissipated in 2-4 hours. However, CC panels should be determined periodically to monitor the heparin withdrawal interval and to assure that the CC does not recur.

While research in blood coagulation has produced an awesome body of intricately complicated information, at the same time coagulationists have provided us with an orderly and comprehensible system of principles and mechanisms in the area of CC which, when properly understood and correctly applied clinically, can be used successfully to treat critically ill patients.

Blood Group Antigens Stored Over Five Months in ACD-Adenine

The preservation of human erythrocyte antigenic specificity following short-term and long-term storage has been reported by investigators consistently since the 1940's. One of the early reports (62) found no change in reactivity of red cells stored at 4°C in acid-citrate-dextrose (ACD) and Alsever's solution for 6 weeks. A definite decrease in titer was noted during the seventh week of storage. The Rh system was used in this study. A modification of Hattersley's (62) technique was employed by Wall, et al (63) who reported that red cells containing A₂ agglutinin were found to be least stable, but suspensions of all blood types employed were satisfactory for use in hemagglutination tests for a period of at least 2 months. One of the more interesting reports is by Cohen, et al (64), using the method of Strumia, et al (65). Erythrocytes were resuspended in a solution consisting of 4% globin, 5% lactose, and 0.25% glucose and stored at 0°-3°C. Following a storage period of 4-10 months, the erythrocyte antigens M, N, P, rh', Rh₀, rh'', hr', rh^w, K, Le^a, Le^b, and Fy^a were compared to fresh cells. One P antigen could not be demonstrated, and reactions of the preserved cells with M and N sera were, although clear, not as strong as the reactions with fresh cells. The Rh₀ antigen retained its reactivity during the 4-10 months' storage period.

Employing a glycerol freezing technique, Krijnen, et al (66) investigated the red cells of four different blood samples containing antigens A, B, M, N, S, rh', Rh₀, rh'', hr', K, Fy^a, Le^a, and Le^b. They found that after 4 months' storage at -79°C, no alterations were detected, by comparing the serological reactions with frozen and with fresh cells of the same persons. Grove-Rasmussen, et al (67), studied the effect on the agglutination properties of blood group antigens in red cells, which had been stored in the frozen state in a mixture of glycerol-citrate and glycerol-lactate. Using the freezing method of Chaplin and Mollison (68), they found no change in the antigenic strength of red cells stored for 20 weeks, when compared to fresh red cells from the same person. The following antigens were studied: Rh₀, rh', rh'', hr', hr'', M, N, S, s, P, Le^a, Le^b, Lu^a, K, k, Fy^a, and Jk^a. Negative controls were included to insure that the agglutination reactions obtained with the frozen red cells were specific.

Nijenhuis (69), using a glucose-citrate solution, a modification of Rous-Turner described by Simmons et al (70), found a decrease in reactivity of all groups, starting as early as a few weeks after beginning of storage. Simmons and his group reported good results, in excess of 6 months, when sterile samples of blood are kept in this fluid at 5°C. Nordqvist et al (71) have described a method for storing erythrocytes up to 17 months. At different times of storage, the agglutinability of the erythrocytes was compared with that of fresh erythrocytes from the same donor. A sufficient agglutination titer was noted after 1 year of storage. At different times, 100 ml of blood were drawn into 22 bottles (500 ml), 16 of which contained 65 ml ACD solution, sodium citrate 2.2 g, acid citricum 0.8 g, glucose 2.45 g per 100 ml ordinary Ringer's solution. The erythrocytes were then separated from the plasma and stored in Ringer's solution at 4°C. Agglutinability was obtained with cells remaining in this solution after more than 1 year. As reported, many erythrocytes tended to be hemolyzed, and the potassium content of the solution increased until a steady state was reached, while hemolysis of the remaining erythrocytes was very slow. However, by immersing the erythrocytes from the beginning in saline with a potassium content higher than that of normal Ringer's solution, hemolysis was prevented to a great extent. The antigens studied were A, Rh₀, hr', and Fy^a.

Comparing saline-suspended and papain-treated erythrocytes under these various experimental conditions, Nordqvist et al (71) found good comparison (one tube decrease in titer) when compared against fresh cells, except for one anti-hr' reaction, which had a saline-suspended titer of 1/2 fresh and 1/1 stored, but 1/256 fresh against 1/32 stored with papain-treated cells. Huntsman et al (72) reported the preservation of red cells, anticoagulated with EDTA and fortified with sucrose, in liquid nitrogen, but did not give a storage period. They report that cells representing A, B, rh', Rh₀, rh'', hr', M, N, K, and Fy groups showed unimpaired reactions after freezing and thawing by their method.

Mears and Marsh (73) employed the glycerol freezing method with cells which had been modified by the enzymes trypsin, papain, and ficin. Following 9 months' storage at -20°C , only papain-treated cells had hemolysis as high as 11%. There was no evidence of polyagglutinability, and loss in titer of antigens Rh_0 , rh'' , rh' , hr' , and hr'' was not significant.

Strumia et al (74) stated that addition of a solution of lactose, dextrose, or a combination of the two sugars prolongs the safe period of exposure to temperatures in the critical range (-3° to -40°), thus exerting a protective effect on red cells of whole blood with ACD. Under optimal conditions, when a mixture of lactose and dextrose is used, $95\% \pm 3$ of the red cell population is recovered intact after freezing and thawing.

Gibbs et al (75) reported that ABO activity of the red cells is preserved over long periods of time when they are stored frozen in liquid nitrogen or glycerol. The use of erythrocytes of the ABO group preserved in dextrose, citrate, sodium chloride, or ACD solutions at refrigerator temperature for comparative work is limited, since only in the period between 6 and 15 days is the agglutinability of all cell types stable. Kevy et al (76) evaluated citrate-phosphate-dextrose (CPD) and reported that the stability of red cell antigens preserved in CPD showed a minimal loss of antigenicity after 28 days.

Sussman and Butler (77) in studies with clotted blood versus blood stored in ACD reported that a slight but definite advantage is obtained by the use of an ACD pilot tube regarding reactivity of some of the antigens on the stored red blood cell. This advantage becomes more marked after 21 days. After this time, the presence of the Kell factor, for example, cannot be identified in blood stored as a clot if weak antisera are used, whereas this factor is detectable by the same weak antisera if the red cell has been stored in ACD solution. The risk in passing such a crossmatch as "compatible" is considerable. This problem will become more acute as better storage formulae for preservation of blood become available. Recent research on adenine and inosine indicates increased blood bank storage potential up to 42 days, with at least 70% posttransfusion viability of the blood, and such findings will certainly require changes in crossmatching techniques.

At present, the demonstration of diminished reactivity of certain antigens on the stored red cell should alert blood bank workers to the need to exercise greater care in performing crossmatching tests on blood that is approaching the present 21-day expiration date. The diminishing reactivity of some antigens on the stored red cell, either as a clot or in ACD solution, is clearly evident in their data. This important fact must be emphasized, especially in the major crossmatch, where serums with low antibody titers may be in contact with red cell antigens of poor reactivity. Minor "roughness" of the crossmatch of such bloods should be received with suspicion.

Bronson and McGinniss (78) described a simple method for the preservation of red blood cells in liquid nitrogen. Their study showed that red cells stored in liquid nitrogen retain their antigen activity for at least 6 months, and after thawing remain active for at least 2 weeks in Alsever's solution.

Strumia et al (79) describe a simple method for red cell preservation modified by lactose-dextrose solution in the frozen state at -93°C . The agglutinability, as measured by a titration of antisera versus fresh and frozen cells, was maintained after periods of storage up to 3 years for the blood factors A, B, rh' , Rh_0 , rh'' , hr' , hr'' , K, k, M, N, and Fy^a . S and s were also still reactive after freezing and thawing.

Purpose. This investigation was carried out to determine:

1. The effect of acid-citrate-dextrose supplemented with adenine free base on the reactivity of human erythrocyte antigens after various periods of storage at $4-6^{\circ}\text{C}$.
2. The stability of red cell antigens in the donor bag and in the segments of the integral donor tubing, which had been mixed with ACD-adenine.
3. The feasibility of using the integral donor tubing segments exclusively (in lieu of pilot tubes) for crossmatching procedures throughout 42 days of storage.

Materials and methods. Donor blood was drawn into ACD solution* and into ACD solution fortified with 33 mg of adenine free base.**

Donors were bled for red cell storage in various preservatives and bled fresh for each series of testing in parallel with stored samples. The storage temperature throughout the study was $4-6^{\circ}\text{C}$. Commercial antisera were titrated for original titer, and frozen in aliquots for parallel and replicate testing. The following protocol was used in the study:

* ACD solution Formula A	
Tri-sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$)	2.2 gm
Citric acid ($\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$)	.8 gm
Dextrose ($\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$)	2.45 gm
Water for injections, to 100 ml	to volume
Volume used for collection of 450 ml of blood	67.6 ml

- **
- 67.5 ACD solution
U.S.P. Formula A with 33 mg adenine contains 15.1m Eq of sodium
0.8 gm citric acid (hydrous), U.S.P.
2.2 gm sodium citrate (hydrous), U.S.P.
2.45 gm dextrose (hydrous) U.S.P.
49.3 mg adenine

1. Determination of the titer by standard, twofold serial dilution of A and B antigens as fresh erythrocytes and after 6 weeks' storage in ACD-adenine.

2. Determination of titer of various blood group antigens in ACD after 4 hours' storage and following storage for 28 weeks.

3. Determination of the titer of A and B antigens and various blood group antigens as fresh cells, and after 7 weeks' storage in ACD-adenine. These were paired samples obtained from split blood donations into ACD and ACD-adenine. The samples were retrieved from bag and/or the integral donor tubing.

4. Determination of the reactivity of red cell antigens stored in ACD-adenine for 49 days for use in a reliable crossmatch procedure.

5. Evaluation of a crossmatch procedure utilizing donor segments of integral donor tubing.

Results. From Table 8, it can be seen that the titers* of the A and B antigens, as well as the subgroups and combined AB antigens, retain their reactivity well after storage in ACD-adenine for 6 weeks, when compared to the titers of the same red cells tested fresh. At 10 weeks, untitered, single tube testing with undiluted serum showed no decline in reactivity, except with an A₂B cell.

TABLE 8
Titer of A and B Antigens -
Fresh and After 6 Weeks' Storage in ACD-Adenine

Antigen		Fresh Cells	Preserved Cells
A ₁		512	512
A ₂		128	128
B		256	256
A ₁ B	A	512	512
	B	128	128
A ₂ B	A	256	256
	B	512	512

*Titer is reported as the reciprocal of the dilution of the last tube showing a 1+ reaction as agglutination.

In Table 9 the titers of Rh, M-N-S, Fy^a, K, and Jk^a antigens are shown when tested 4 hours after storage and after 28 weeks of storage. These titers reveal, as previously reported, that this length of storage results in an average one or two tube decrease in antigen reactivity when ACD preserved cells are stored at 4-6°C.

TABLE 9
Titer of Various Blood Group Antigens in ACD
After 4 Hours' Storage and Following Storage for 28 Weeks

Antigen	ACD (4 hrs)	ACD (28 wks)
Rh ₀	64	32
Fy ^a	16	8
M	8	4
N	8	4
S	8	4
Jk ^a	16	8
K	64	32

Table 10 shows the results of storage on red cell antigens of various donors drawn as split donations in ACD and ACD-adenine versus fresh cells. The titers were performed in duplicate and these were scored.* The A-B antigens retain their reactivity well when compared to the titer of fresh cells and when ACD-adenine is compared with ACD. Similarly, good reactivity is seen in the other blood group systems which were tested in parallel with the A-B antigens. These include Rh₀, K, Jk^a, M-N-S, Fy^a, and Le^a. The titrations reveal the reactivity of these red cell antigens when tested against strong antisera and against weak antisera. The Kell antigen can be seen reacting well with weak antisera against fresh red cells and 49-day-old red cells stored in ACD and ACD-adenine.

Crossmatch for transfusion. The main consideration in this study has been that of suitability of red cells and plasma following storage in ACD-adenine for 6 weeks for use in a safe crossmatching procedure. The studies have demonstrated that cells and plasma from the bag and integral donor tubing segments are satisfactory for all crossmatching purposes, in that false positives do not occur, denaturation or deterioration of plasma antibodies does not occur, and, finally, incompatibility can be observed with all systems tested, with low concentration of antibody.

* Scoring technique: 4+ = 12 3+ = 10 2^S = 8 2 or 2^W = 5 1^S = 3
1 or 1^W = 2 ± = 1

TABLE 10
Reactivity as Titer/Score of Fresh and Stored Red Cells

Antigen	Fresh	ACD (7 Wks)	ACD+AD (7 Wks)
A ¹	256/87	256/83	256/85
A ²	64/67	64/67	64/66
B	256/80	256/91	128/83
Rh ₀	64/50	64/50	64/49
Fy ^a	32/35	32/43	32/37
M	8/26	8/24	8/23
N	2/5	2/2	2/3
S	8/22	8/26	8/25
K	512/75	512/73	256/66
Xg ^a	16/10	16/11	16/14

Technique for crossmatch utilizing donor tubing segments. Under conditions of extended storage, the pilot tube becomes difficult to work with due to various factors, such as: drying, hemolysis, contamination, and breakage. In addition, there is the time-proven fact that pilot tubes are subject to clerical or technical mix-up, and are regarded by many laboratories as "wild" tubes. The integral donor tubing, which is firmly attached to the donor bag, has the advantages of segmentation, sterility, numerical identification corresponding to the donor bag number, and, finally, preservation of the erythrocyte antigens is enhanced by the ACD-adenine solution.

Obtaining cells and plasma (80). Figure 12 shows the numbered segments, cell-plasma interface, and the unit of whole blood for crossmatch. The segment is detached by cutting with scissors as shown in the figure.

Figure 13 illustrates the use of a 12 x 75 mm centrifuge tube to spin down the cells and provide a cell-plasma interface. Normal sedimentation may have already achieved this separation. The tube may be filled with saline to further support the segment. Figure 14 depicts the segment being clamped off by a hemostat at the cell-plasma interface. Some workers simply cut with scissors.



Figure 12. Detachment of numbered segment.

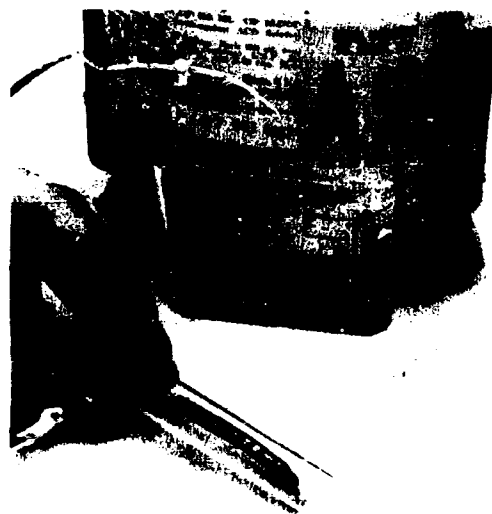


Figure 13. Centrifuge tube to spin down cells.



Figure 14. Clamping of segment at cell-plasma interface.

Figure 15 demonstrates the preparation of a saline-cell suspension by merely squeezing the segment to obtain the required cells.

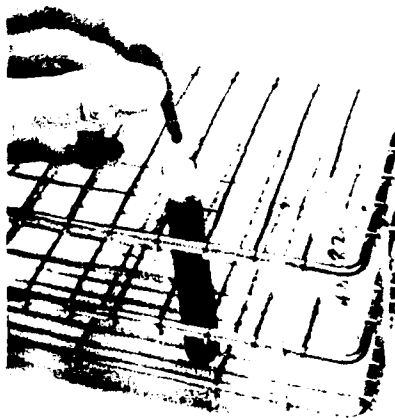


Figure 15. Preparation of saline-cell suspension.

Figure 16 gives an alternate and preferred method of obtaining a controlled amount of red cells to prepare a saline-cell suspension, by use of an applicator stick.

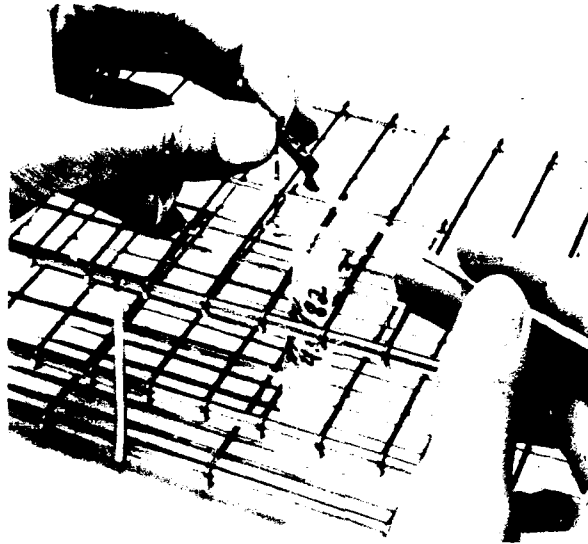


Figure 16. Use of applicator stick for controlled saline-cell suspension.

The steps required in the established crossmatch protocol are then carried out. Special note should be made of the following:

1. Complement necessary for certain antigen-antibody reactions can be supplied by the recipient's fresh serum sample.

2. If fibrin forms in the major side of the crossmatch, the donor red cells should be washed four times with saline.

3. If fibrin forms in the minor side of the crossmatch, the recipient's red cells should be washed four times with saline.

Summary. Red cell antigens of the A-B system retain their reactivity in excess of 42 days when stored in ACD-adenine at 4-6°C.

The Kell antigen is detectable at 42 days' storage in ACD-adenine with a weak antisera, or in plasma with a low titer anti-Kell.

The integral donor tubing segments of ACD-adenine stored blood may be used exclusively (in lieu of pilot tubes) throughout a 42-day storage period for safe crossmatching procedures.

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